

AGE DETERMINATION AND POPULATION BIOLOGY
OF THE FLORIDA MANATEE,
TRICHECHUS MANATUS LATIROSTRIS

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1993

To manatees, for some of the best years of my life.

ACKNOWLEDGEMENTS

Throughout this project Tom O'Shea was more than advisor, he was a mentor, participating in all steps of my graduate life. I thank him for that and for believing in my project. Steve Humphrey provided guidance during the whole process of research and writing. Roger Reep provided space, microscopes, the image-analysis system at his laboratory and good poetry. I thank Mel Sunquist and John Eisenberg for encouragement. Dr. C. Lanciani helped with concepts and calculation of population parameters. Dr. L. E. Eberhardt offered valuable comments on an earlier version of Chapter 4.

Special thanks go to Dr. Aleta Hohn (Southwest Fisheries Center) for guidance in developing the age-determination technique and interpreting an initial series of histological slides. Dr. Douglas Jones (Florida Museum of Natural History) allowed the use of the rock saw for rough-sectioning of samples. Dr. Anthony Randazzo (Geology Department, University of Florida) prepared samples for viewing under polarized light. Daniel Williams lent his expertise in computer and image analysis of specimens. Dr. Daryl Domning provided guidance and suggestions at the beginning of the project, and the sample of Steller's sea

cow earbone. The Florida Department of Natural Resources allowed access to manatee mortality records between 1985 and 1991. Donna Banowetz compiled reproductive data from salvage records from 1985 on. I thank Melanie Davis and her staff for numerous articles obtained through interlibrary loan.

The people at the Sirenia Project provided a unique atmosphere of professional cooperation and friendship that was delightful. Patty Furr solved many administrative and software-related problems with promptness and good humor. I thank Howard Kochman for invaluable statistical help, introduction to the "data step" and witty comments on lab life. KB shared endless night-shifts at the lab, margaritas and bitnet messages.

I gratefully acknowledge the U.S. Fish and Wildlife Service and the Florida Department of Natural Resources, as well as all the cooperators who have participated in the manatee carcass salvage program for providing samples and collecting data used in this study, particularly Cathy Beck and Robert Bonde of the Sirenia Project, Dr. Scott Wright, David Carson, Mark Ludlow, and James Valade of the Florida Department of Natural Resources, Dr. Daniel Odell of Sea World of Florida, and Antonio Mignucci of the Caribbean Stranding Network. I thank curators and curatorial staff of the following institutions for granting access to specimens: the Burke Memorial Washington State Museum, University of

Washington, Seattle; the California Academy of Sciences, San Francisco; the Carnegie Museum of Natural History, Pittsburgh; the Florida Museum of Natural History, Gainesville; the Los Angeles County Museum of Natural History, Los Angeles; the Museum of Natural and Cultural History, Oklahoma State University, Stillwater; the Museum of Southwestern Biology, University of New Mexico, Albuquerque; the North Carolina Museum of Natural History, Raleigh; the Oklahoma Museum of Natural History, University of Oklahoma, Norman; the Orman J. Smith Museum of Natural History, Albertson College of Idaho, Caldwell; and the U.S. National Museum of Natural History, Washington, D.C.

Policies to inject wild and captive manatees with tetracycline were initiated in 1981 by the Sirenia Project under the guidance of Drs. Robert Brownell, Jr., and Galen Rathbun and were carried out with the assistance of staff at Sea World and Miami Seaquarium. Tetracycline administration and salvage of specimens was authorized under Federal Wildlife Permits PRT-2-4405, PRT-2-6983, PRT-2-8430, and PRT-684532 issued to Dr. Brownell. This study was partially funded by the U.S. Marine Mammal Commission (Contract T 6223918-1), the National Fish and Wildlife Foundation (Project no. 89-080), and the Sirenia Project, National Research Ecology Center, U.S. Fish and Wildlife Service. I was supported through scholarships from the Brazilian

government (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Conselho Nacional de Pesquisas) and a research assistantship from the Florida Cooperative Fish and Wildlife Unit, at the University of Florida.

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KEY TO SYMBOLS

BC-000	Brevard County, FL
BS-000	Blue Spring State Park, FL
CAS 00000	California Academy of Sciences, San Francisco
CIMNH 00000	College of Idaho Museum of Natural History, Caldwell
CM 00000	Carnegie Museum of Natural History, Pittsburgh, PA
CR-000	Crystal River, FL
FDNR	Florida Department of Natural Resources, St. Petersburg
KDL-0000	Kissimmee Diagnostic Laboratory, FL
LACM 00000	Los Angeles County Museum of Natural History
M-000	U.S. Fish and Wildlife Service, Sirenia Project, Gainesville
M-00-00	Rosenstiel School of Marine and Atmospheric Science, University of Miami
MJAV-0000	Florida Department of Natural Resources,
MNE-0000	Northeast Field Station, Jacksonville
MNW-0000	Florida Department of Natural Resources, Northwest Field Station
MSB 00000	Museum of Southwestern Biology, University of New Mexico, Albuquerque
MSE-0000	Florida Department of Natural Resources, Southeast Field Station, Miami and Tequesta
MSQ-000	Miami Seaquarium
NCSM 00000	North Carolina State Museum, Raleigh

NEP-ST-000	Caribbean Stranding Network, Puerto Rico
NM 00000	National Museum of Natural History, Smithsonian Institution, Washington D.C.
OMNH 0000	Oklahoma Museum of Natural History, University of Oklahoma, Norman
OSU 0000	Oklahoma State University, Stillwater
SWFTm0000X	Sea World of Florida, Orlando
UF 00000	University of Florida, Florida Museum of Natural History, Gainesville
UWBM	University of Washington Burke Museum, Seattle

Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

AGE DETERMINATION AND POPULATION BIOLOGY
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By

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May 1993

Chair: Dr. Stephen R. Humphrey
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Major Department: Forest Resources and Conservation
(Wildlife and Range Sciences)

Florida manatees (*Trichechus manatus latirostris*) are at risk due to modern stresses on the population, primarily from boat traffic and habitat alteration. Life history and population studies to understand the consequences of such stresses have been hampered by the lack of a method of age estimation. Skeletal material of 16 known-age, minimum known-age, or tetracycline-marked Florida manatees was prepared using modifications of histological techniques employed in age-determination studies of dolphins. Results consistent with known age, minimum known age or tetracycline-labeling were obtained only from the dome region of the periotic bone. Age-specific aspects of mortality and reproduction were evaluated for 1,212 manatee specimens collected between 1976 and 1991. Approximately 59

layers were found in the periotic dome of one manatee of unknown age, and several had growth-layer-group counts ranging from 21-39. Sexual maturation can occur between 3 and 4 years of age. At any one time 33% of the mature females were pregnant, indicating an average calving period of 3.0 years. Fecundity remained relatively unchanged (0.24 female offspring/adult female/year) from age of first parturition throughout life. Half the carcasses belonged to age classes 0, 1 and 2, and average age was low (5.7 years). Survival rate was low among the very young, increased up to age class 4, and remained constant (89.6%) from age class 4 to at least 39. The manatee survivorship curve is consistent with the type expected in a long-lived mammal, but lacks a plateau through middle age. The steeper gradient is attributed to exacerbated levels of adult mortality represented by the constant threat of collision with watercraft. Death from intense cold affected mostly juveniles, but the effect of boat strikes was constant across age classes. Computer simulation (VORTEX51) projected a 44% chance of persistence over 1,000 years for a stationary (rate of increase $r = 0$) manatee population. The current situation allows no margin of error. If increasing numbers of boats result in more deaths, the manatee population will tend toward extinction. Only cultural change will prevent this outcome.

CHAPTER ONE

INTRODUCTION

Manatee Biology

The West Indian manatee (*Trichechus manatus*) is a large aquatic mammal inhabiting tropical waters of the New World Atlantic. Differences in cranial morphometrics confirmed a differentiation into two subspecies, isolated by the climatic and hydrological barriers of the Gulf of Mexico and the Straits of Florida (Domning and Hayek, 1986). The Florida manatee (*T. manatus latirostris*) is mostly confined to Florida during the winter, with comparable numbers on either side of the peninsula (Hartman, 1974; Irvine and Campbell, 1978), but individuals may disperse further north during the warmer months (Moore, 1951; Rathbun et al., 1982). The Antillean manatee (*T. manatus manatus*) ranges from the Gulf of Mexico to northeastern Brazil, with discontinuities due to lack of habitat or previous decimation by hunting activities (Lefebvre et al., 1989; Thornback and Jenkins, 1982).

Manatees are herbivores and ingest a variety of species of aquatic plants (Best, 1981; Hartman, 1979; Ledder, 1986). They inhabit inland rivers or coastal and estuarine waters

with access to freshwater sources and deep-water canals (Hartman, 1979). Manatees are physiologically constrained from spending prolonged periods in waters below about 20°C by their low metabolic rate and high thermal conductance (Irvine, 1983). Whitehead (1977) gathered records supporting a distribution of *T. manatus* coinciding with the 24°C mean annual isotherm. During cold periods Florida manatees seek warm-water sources, often engaging in long-distance seasonal migrations (O'Shea and Ludlow, 1992; Reid and O'Shea, 1989) to aggregate around natural springs or industrial warm-water effluents (Shane, 1984). Manatees have no natural predators except for man (Rathbun, 1984).

The Manatee Carcass Salvage Program

A cooperative multiagency effort was initiated by the University of Miami and the U.S. Fish and Wildlife Service in 1974 to recover all dead manatees found along the coasts of the state. Lack of resources at the inception of the program caused carcass and data collection not to be fully operational until late 1975 (Campbell and Irvine, 1981). Between 1974 and 1985, the U.S. Fish and Wildlife Service Sirenia Project, in Gainesville, coordinated the collection of carcasses and assignment of death categories. In late 1985, the Florida Department of Natural Resources, in St. Petersburg, assumed the coordination of the program. The main cooperating agencies in the recovery and necropsy

process were the Florida Marine Patrol, U.S. Army Corps of Engineers, University of Miami, and Sea World of Florida.

Reported carcasses were recovered whole whenever possible. Materials collected included the skeleton, tissue for histology, reproductive tract, stomach contents, and parasites. If allowed by the degree of decomposition, a necropsy was performed to determine cause of death. In virtually every case, even when no soft tissue remained, scientists collected the skull of the deceased manatee; whenever the carcass was reasonably intact scientists minimally recorded total body length (in cm) and sex of the manatee. Salvage procedures appear in Bonde et al. (1983). Between April 1974 and December 1991, 1,936 carcasses were handled (855 females, 959 males, and 122 of sex unknown). Specimens included neonates as well as mature individuals, with a range of sizes between 30 and 411 cm total length.

Current Threats, Status, and Perspectives

Accounts on population abundance over time have been contradictory; however, there is no evidence that manatees were ever abundant in historic times (O'Shea, 1988). No reliable technique exists for estimating population size (Lefebvre et al., 1992; Packard, 1985a, Packard et al., 1985). The first population estimate for manatees was based on a summer aerial survey of both coasts of Florida, the St. John's River and the Florida Keys, conducted in 1973

(Hartman, 1974). The original count of 255 animals, modified by a subjective correction factor for water clarity, produced a rough estimate of 750 to 850 manatees. In 1976 the first winter aerial survey was conducted over warm-water sites, yielding a count of 738 manatees (Irvine and Campbell, 1978). Brownell et al. (1981) translated these into a minimum population estimate of 800 to 1,000 Florida manatees, and the U.S. Fish and Wildlife Service and Florida Department of Natural Resources revised the later estimate into a minimum of 1,200 animals (O'Shea, 1988). Following cold fronts during the winters of 1991 and 1992 the Florida Department of Natural Resources conducted statewide synoptic surveys, with the following results: 1,268 (January 1991), 1,465 (February 1991), and 1,856 (January 1992) (Ackerman, 1992). Like the previous estimates, these numbers have no statistical confidence intervals and represent a minimum estimate of the Florida manatee population. Despite increasing values produced in these recent surveys and revised estimates, numbers must be interpreted with caution because, as suggested by Eberhardt (1982), they can reflect improvements in survey methods as well as possible changes in population size.

The total number of manatees found dead has averaged 140 in the past 10 years, and 168 in the last 5 years. This last figure corresponds to almost 10% of the minimum population estimate and could represent mortality levels

incompatible with population maintenance. However, this relationship is uncertain because of the lack of suitable estimates of both carcass recovery rates and population size. Human-related causes accounted for the largest proportion (33 to 53%) of all deaths with identifiable causes in the past 10 years (1983-92). The species' habitat requirements and naturally slow movements render it especially vulnerable to encounters with boats, the most important cause of mortality, because it has increased 10.3% yearly since 1976 (Ackerman et al., 1992). Analysis of mortality patterns over the years 1976-81 revealed a greater incidence of collision with boats on the east coast of Florida (80%), particularly the northeastern portion (O'Shea et al., 1985). Other causes of mortality include crushing in canal locks and flood gates (Odell and Reynolds, 1979), entanglement and ingestion of debris and fishing equipment (Beck and Barros, 1991), perinatal factors (O'Shea et al., 1985), natural catastrophes (O'Shea, 1988; O'Shea et al., 1991) and disease (Buergelt and Bonde, 1983; Buergelt et al., 1984). The Florida manatee population has relatively high levels of genetic variability (McClenaghan and O'Shea, 1985).

Manatees in Florida have been granted protection for almost a century (Hartman, 1974) but the population is considered endangered by what is judged to be excessive mortality and habitat limitations. The species is

classified as endangered under the U.S. Endangered Species Act of 1973 and receives protection under three additional recent pieces of legislation: the U.S. Marine Mammal Protection Act of 1972, the Florida Endangered and Threatened Species Act of 1977, and the Manatee Sanctuary Act of 1978. Conservation measures implemented in the last decade include the designation of sanctuaries, boat speed limit zones, regional protection plans, and educational campaigns (O'Shea and Ludlow, 1992).

Florida manatees have shown plasticity and adaptability through taking advantage of protected areas, warm-water refuges, proliferation of exotic aquatic vegetation, and sources of fresh water (O'Shea and Ludlow, 1992; Rathbun et al., 1990). However, potential threats are very numerous and significant, not the least of them being the rapid rate of coastal development and consequent habitat modification. The Florida human population grew by 32.8% between 1980 and 1990, and is projected to increase by 40% in the next 20 years (Bureau of Economic and Business Research, 1991). Most of the growth in the past decade (86.8%) was due to immigration, which translates into approximately 760 new residents a day. A large proportion of those settle in coastal areas, where boating activities play an important recreational role. The number of registered pleasure boats increased by 3.8% since 1981 (Ackerman et al., 1992). The constant influx of new residents to Florida and the

continuing increase in boat registrations indicate that boat-related mortality may continue to rise. Other factors that may become prominent in the future are the closing of industrial sources of warm water, contamination of waterways, and potential for spread of diseases.

A Manatee Recovery Plan was developed in 1989 by a Recovery Team from governmental and nongovernmental institutions. The Recovery Plan details a series of activities relative to protection, research, and management to be conducted with the goal of downlisting the species from endangered to threatened status (U.S. Fish and Wildlife Service, 1989). Some of the primary objectives of the recovery plan refer specifically to better understanding of population biology and call for the construction of population models as a means of monitoring population status.

Population Analysis

Long-term population studies of large mammals are a rarity, especially for endangered species; a few exceptions include studies on African elephants *Loxodonta africana* (Buss, 1990; Douglas-Hamilton, 1987), Pribilof fur seals *Callorhinus ursinus* (Eberhardt, 1981; Smith and Polacheck, 1981) and grizzly bears *Ursus arctos* (Craighead et al., 1974; Suchy et al., 1985). The first effort at an integrated analysis of manatee demography was conducted in

the early 1980s (Packard, 1985b). This work consisted of age-structured models based on the Leslie matrix approach. The models suggested a low maximum potential rate of increase, with a small range of positive values, but not discounting the possibility of a declining population. However, the database on manatee demography at that time was very preliminary. Since then many more data have been acquired on the biology of the species. In particular, the manatee carcass salvage program is still in operation and has produced a wealth of information on manatee biology.

My study describes the development of a technique for age determination and its application to specimens collected from 1974 to 1991. I also derive population parameters on mortality and reproduction based on this material, and apply these parameters to both a life table approach and a population viability analysis. The latter computer simulations identified the relative importance of the factors affecting the population and assessed the strength of possible management alternatives.

All specimens examined in this study came from manatees that died for reasons unrelated to this study and were opportunistically recovered through the manatee carcass salvage program. No manatees were sacrificed for purposes of this research.

CHAPTER TWO

DEVELOPMENT OF AN AGE DETERMINATION TECHNIQUE
IN FLORIDA MANATEES

Introduction

Human recreational activities and coastal development have contributed to habitat loss and rising mortality for Florida manatees (*Trichechus manatus latirostris*) (U.S. Fish and Wildlife Service, 1989; U.S. Marine Mammal Commission, 1990). Long-term survival of manatees in Florida depends on management policies based on compromise in terms of human activities, as well as on sound knowledge of manatee population dynamics. Key elements of Florida manatee population biology, such as longevity, population age structure, and age-related aspects of reproduction and mortality cannot be fully understood without a reliable means to determine age of individual animals. The revised Florida Manatee Recovery Plan (U.S. Fish and Wildlife Service, 1989) calls for the development of such techniques and their application to population studies.

Long-term studies based on salvaged manatee carcasses in Florida have produced extensive data on biological parameters as well as samples for future analyses. My study took advantage of the fact that over 1,200 skulls from

salvaged manatee carcasses were available for study. This chapter describes the use of growth-layer groups (GLGs) in bone as an age determination technique for Florida manatees. The objectives were to (1) review current techniques for marine mammal age determination in order to select appropriate methodology for manatees, (2) examine a variety of manatee bones for the presence and distinctiveness of growth layers, and (3) determine if growth-layer counts correspond with chronological age or time intervals since tetracycline administration. Tetracycline produces a fluorescent mark in living bone tissue at the time of injection (Frost et al., 1961), allowing calibration of subsequent layering. Domning and Myrick (1980) first successfully showed the presence of a label in the ribs of an Amazonian manatee injected with a tetracycline antibiotic. More detailed and refined analysis of other topics concerned with manatee age-determination and age-related aspects of manatee biology are presented in the following chapters.

Marine Mammal Age Determination Techniques

Periodic growth marks in skeletal tissues of mammals were recognized as criteria for age assessment as early as 1950 (Scheffer, 1950). Excellent reviews on this topic have been produced subsequently (e.g. Klevezal' and Kleinenberg, 1969; Morris, 1972; Perrin and Myrick, 1980). Techniques

that have been developed to produce indices of relative and chronological age include use of the following: morphometrics, body weight, eye-lens weight, baculum size, skull and epiphyseal suture ossification, tooth succession, tooth wear, inner diameter of teeth, annual ridges on the tooth roots, changes in cell composition of bone marrow, external features (e.g., pelage, secondary sexual characteristics, skin scars), ovarian scars, loss of elasticity of collagen fibers, amino-acid racemization in teeth and eye-lens nuclei, and growth-layer-group counts in horns, claws, earplugs, baleen, teeth, and bone. However, many of these methods are not applicable to manatees. For example, manatees lack bacula, baleen, earplugs, pelage, horns, and obvious secondary sexual characteristics. Counting of corpora lutea in ovaries cannot provide age estimates because the species is polyovular (Marmontel, 1988). The manatee skeleton is characterized by pachyostosis: bones are exceedingly massive and dense, and the medullary cavity is lacking in long bones and ribs (Fawcett, 1942a, 1942b). This condition has deterred analysis of bone for manatee age determination studies because of difficulty in tissue preparation and analysis.

Among other marine mammals, incremental lines corresponding to annuli in the teeth have been used to determine age in pinnipeds (Laws, 1952; Lydersen and Gjertz, 1986; Payne, 1978; Scheffer and Myrick, 1980), and

odontocetes (Goren et al., 1987; Hohn et al., 1989; Perrin and Myrick, 1980; Scheffer and Myrick, 1980). This approach has also been followed in the dugong (*Dugong dugon*), where the permanent, tusk-like incisor teeth possess growth-layer groups that are deposited annually (Marsh, 1980; Mitchell, 1978). Unlike dugongs, manatees lack incisors; similar to elephants and in contrast to other mammals, manatees present a unique dentition, with the production of molars that are replaced by forward projection from the back of the jaw (Domning and Hayek, 1984; Laws, 1966). Manatees are unique, however, by having an indeterminate number of supernumerary molars that are continually replaced throughout life (Domning and Hayek, 1984). This renders teeth inappropriate for age-determination studies, except in young individuals.

Linear body dimensions may indicate age in manatees during the first 5 to 6 years of life, and total body length has been used to categorize individuals as calves, subadults or adults (O'Shea et al., 1985). However, this measure does not permit assessment of exact age. Free-ranging Florida manatees measured periodically from birth to nine years of age show increasing size over this period, but year classes overlap broadly in size and the rate of growth slows after early years (O'Shea and Reep, 1990).

The ossification of flipper bones also has potential utility as an age estimator. Radiology of flippers has been used to classify Florida and Amazonian (*T. inunguis*)

manatees into general categories of relative age (Best, 1982; Hensen, 1986; Ribeiro and Best, 1984). The epiphyses of long bones and cranial sutures in manatees also are slow to ossify and may provide a means to determine relative age of specimens (Fawcett, 1942a, 1942b; O'Shea and Reep, 1990; Pocock, 1940). However, these and all other potential techniques for relative age determination require knowledge of chronological age or known-age standards for calibration and evaluation prior to application to manatees.

The most promising technique for absolute age determination in manatees, and for validation of various criteria relative to age of an individual, may be the quantification of growth-layer groups in bones. Bone growth occurs due to external thickening of the periosteal zone to support additional stress. The bone tissue of the periosteal zone in mammals consists of a mineralized fibrous substance with numerous cellular elements, the osteocytes (Klevezal' and Kleinenberg, 1969). Porous or reticular bony tissue without layers is formed in large, fast-growing animals; compact periosteal bone is found in small animals and in large, slowly-growing animals (Klevezal' and Kleinenberg, 1969). There are variations in thickness, number of layers, and rate of deposition among and within species.

Originally described for poikilothermic vertebrates (Peabody, 1961), growth-layer groups in bone have been

demonstrated to correspond to annuli in some species of mammals (Klevezal' and Kleinenberg, 1969; Laws, 1960). However, bone is a dynamic tissue capable of undergoing remodeling. The assumption that growth layers are annual and that their total number corresponds to true age must be carefully validated by known-age material. The prior absence of known-age specimens and the pachyostotic nature of sirenian bones (requiring more elaborate techniques and equipment for specimen preparation) have heretofore delayed evaluation of bone growth-layer groups as an indicator of age in manatees. However, in a preliminary study, the National Marine Fisheries Service (NMFS) Southwest Fisheries Center examined a series of Florida manatee bones from 12 individuals collected by the U.S. Fish and Wildlife Service prior to 1980 (Myrick and Kelly, 1981). Results were promising in that growth-layer groups were present, but their meaning was uncertain because they were not validated with known-age or tetracycline-marked specimens. Similarly, Best (1982) and Domning and Myrick (1980) found growth-layer groups in earbones and ribs during preliminary studies of Amazonian manatees.

Skeletons from several known-age or tetracycline-labeled Florida manatees have been collected since these pilot studies were conducted. The objective of this study was to use these specimens, as well as a larger sample of individuals of unknown age, to more conclusively determine

the feasibility of developing age-determination methods for Florida manatees based on growth-layer groups in bone.

Materials and Methods

Characteristics of Specimens Examined

Skeletons or partial skeletons of 16 known-age, known minimum-age or tetracycline-marked manatees (Table 2.1) were used to validate age-determination techniques. Known-age specimens included captive-born manatees as well as individually recognizable members of a wild population from the upper St. Johns River at Blue Spring. The latter population has been intensively studied by the U.S. Fish and Wildlife Service Sirenia Project, Florida Park Service, and cooperators for the past 14 years. Individuals are recognized based on unique scar patterns that are documented by extensive photographic records. Other material used for validation of age criteria included 9 manatees injected with tetracycline in the field or at oceanaria. Additionally, earbones from 1,196 carcasses of unknown age and without tetracycline labels were also examined (Appendix A). These included most of the individual carcasses recovered by various institutions in the southeastern United States between August 1974 and December 1991 that were available through local agencies and museums. This larger sample allowed further comparisons of the suitability of different bones for age determination and allowed the examination of

bone layering over a wider range of body sizes and other variables. In a few of these cases age-related information was also available. Animals salvaged in Puerto Rico and Belize, representing the Antillean subspecies (*Trichechus manatus manatus*), were treated as a separate sample (see Growth layers in other sirenians and Table 2.2). One specimen of Steller's sea cow (*Hydrodamalis gigas*) was also processed.

Museum catalog numbers are used to identify specimens. Carcass salvage numbers are used in cases where specimens have not yet been accessioned into museum collections. For cross-reference, field or oceanarium identification numbers and names for individually known manatees are also given (Appendix B). Body size measurements given for carcasses are total body lengths (Bonde et al., 1983).

Three terms are used to qualitatively denote relative age of manatee specimens: calf, subadult, and adult. Calves are considered to be dependent, nursing young; subadults are weaned manatees that are reproductively immature; and adults are reproductively mature manatees. Field studies on recognizable individuals have shown that calves nurse for one to two years, and that sexual maturity occurs at ages ranging from three to eight years (Hartman, 1979; Rathbun et al., in manuscript; U.S. Fish and Wildlife Service Sirenia Project, unpublished data). These terms are used with confidence to describe specimens that have known histories

based on field studies or records in captivity, and those for which necropsy records provide data on reproductive condition or other indices of maturity (see Chapter 3). Where such ancillary information is not available criteria of body size defined by O'Shea et al. (1985) are used. These criteria define all manatees less than 175 cm total length as nursing calves, all manatees greater than 275 cm as adults, and most of those in the intermediate range as subadults.

Tetracycline Marks

Nine of the sixteen animals listed in Table 2.1 received tetracycline injections while living. Time between administration and death varied between 7 and 131 months (Table 2.1). Tetracycline binds with calcium and is incorporated into tissues that are being laid down or that are active at or shortly after (within 48 hours) the time of administration, including walls of Haversian canals and osteocyte lacunae, and periosteal surfaces (Bevelander et al., 1961; Dunstan and Evans, 1980; Frost, 1969; Frost et al., 1961; Yagi et al., 1963). Under visible light tetracycline-marked bone exhibits a yellow-brown color, and under ultraviolet light (360 nm wavelength) it produces a characteristic yellow-gold fluorescence (Bevelander et al., 1961; Frost, 1983; see Fig. 2.21). The total thickness of the label depends on the daily rate of addition of newly

mineralized bone matrix, and the length of the administration period, but not on the amount of antibiotic given per unit of time (Frost et al., 1961). Frost et al. (1961) suggested that labels may fade after a certain number of years, although not completely. Labels may also be lost under situations of bone resorption or remodeling (Frost et al., 1961). Tetracyclines are non-toxic in small amounts (Spinage, 1973). Because of its utility in calibrating growth-layer groups, the U.S. Fish and Wildlife Service Sirenia Project and cooperators began a program of routinely injecting captive manatees and wild individuals from selected areas with tetracycline in 1981. Sixty-nine manatees have been marked through December 1992. Liqueamycin LA-200 oxytetracycline (Pfizer)¹ was administered for labeling bone at a dosage of 30 mg/kg to 19 wild manatees caught by net at Blue Spring. Injection was intramuscular at multiple sites (up to 15 ml/injection site) in the dorsal peduncle area. This dosage and pattern of administration were also routinely followed for manatees held captive for rehabilitation. Further, specimen UF 20757, a wild-caught female held at Sea World, received oral and intravenous doses of tetracycline during treatment for disease.

¹The use of any trade names in this document does not constitute an endorsement of any product, product line, or company.

Sample Preparation

Methods developed by biologists at the National Marine Fisheries Service (NMFS), Southwest Fisheries Center (SWFC) in La Jolla, California, for examination of teeth and bone of various dolphin species (Hohn, 1980; Hohn et al., 1989; Perrin et al., 1976, 1977) were employed and modified for manatee bone investigations. A detailed protocol for specimen preparation procedures (Appendix C) was developed following consultation visits to the SWFC. A brief synopsis of the methods follows: nearly all bones examined were stored dry in air between death and processing. The earbone of one specimen (M-396), however, had been kept in 10% neutral buffered formalin since 1984, and those of SWFTm-9002B and MJAV-8921 were formalin-fixed at necropsy and kept in fixative until examined. These samples provided excellent material for microscopic examination in comparison with many of the samples stored dry. All earbones collected since late 1989 were fixed in formalin and maintained in that medium or transferred to 70% alcohol allowed easy handling and production of unbroken histological sections.

Bones were rough-sectioned on a rock saw to provide a holding surface for fine sectioning. A 4-mm section was cut for decalcification and histological preparation. Samples were fixed in formalin overnight prior to decalcification (in the case of dry bones). Specimens were then decalcified

in RDO¹ (Apex Engineering Products, Appendix C) and rinsed thoroughly. A freezing microtome was used to produce 40 μ m sections, which were subsequently stained in Mayer's hematoxylin (modified by Grue, see Appendix C). Thin sections were mounted in glycerin. "Thick", untreated, sections for fluorophore examination were mounted directly on the slide.

Histological bone sections were viewed under transmitted light. An Olympus¹ BH-2 photomicroscope equipped with an Osram HBO 100 W/2 super pressure mercury lamp was used for viewing of fluorescence sections. All sections were first examined by eye against natural light and then at low power (10x final magnification) for an overall view of the general pattern. Bone structure of fetuses and neonates was generally examined under 10 or 20x. The majority of the layers of small and medium-sized animals could be resolved under 100x. Fine detail between very close adhesion lines on the periosteal layer of bones from large, presumably old manatees required 400x. At higher power, the glycerin film where the section was mounted interfered with the reading.

"Thick" sections (approximately 140 μ m) were made of tetracycline-labeled bone for examination under ultraviolet light. Calibration of rate of growth-layer group formation for tetracycline-labeled specimens was obtained by comparing the stained section with the thick section of the same bone.

A Microcomp¹ morphometry program (Southern Micro Instruments) run on a Hewlett-Packard¹ personal computer allowed accurate measurement and comparison of growth layers in stained and fluorescence sections. Multiple measurements ($n \geq 10$) were made in the middle 1/3 of the dome section of the earbone. The distance from the tetracycline label to the edge of the periosteal layer was measured for the thick section. Subsequently, the number of bone growth layers contained within that distance in the stained section was recorded to compare with the time interval elapsed between tetracycline injection and death. Edge effects rendered the task of finding the tetracycline label difficult. After acquiring experience, fluorescent lines were more easily identified. They were best viewed under final magnification from 40 to 200x. Lower magnification produced too dark an image without good resolution, whereas higher magnification rendered the image blurry.

An image analysis system (IBAS)¹ was used to analyze, measure, enhance, and photograph growth layers. Both stained and fluorescence sections were photomicrographed to allow more detailed examination and comparison. Black-and-white prints of the stained sections were produced from Kodak¹ Technical Pan Film 2415 (ESTAR-AH Base) shot at 50 ASA and developed at moderate contrast. Kodak Ektachrome P800/1600 film was used for fluorescence sections.

Definitions and Enumeration of Growth-Layer Groups

A *post hoc* method of defining growth-layer groups for age-class estimation was developed based on initial findings presented in detail in the results section of this chapter. This method also is supported by studies of other species. In dentine and cement of other mammals (Klevezal' and Kleinenberg, 1969; Perrin and Myrick, 1980) a broad and weakly stained band followed by a narrow and intensely colored line represents an annual layer. In bones of humans and laboratory animals, the wide bands represent periods of active bone growth, whereas narrower lines represent reductions in apposition of bone tissue (Sissons, 1949, 1971; Weinmann and Sicher, 1947). In the stained sections of decalcified bone tissue prepared for this study, broad, opaque bands, rich in cells and well vascularized ("zones"), also stained less intensely than the thinner, bordering hematoxylinophilic lines (Castagnet's "arrest lines of growth" [Castagnet, 1974] or adhesion lines) that separate them. Hematoxylin stains well-calcified tissues more intensely (Schour and Massler, 1949). Adhesion lines lack fibrils and consist of an adhesive substance impregnated with calcium salts and are more calcified than the collagen of bone (Amprino and Engström, 1952; Weinmann and Sicher, 1947). Based on the material available for this study, a broad zone followed an adhesion line is considered to be a bone growth-layer group representing an annual period. The

difference in distinctiveness of the first, and sometimes the second bone growth layer found in manatee periosteal material is also corroborated by studies for cement, dentine, and bone of other species (Laws, 1953, 1958; Mansfield, 1958). Zones deposited during the first years have a disordered structure but become progressively more organized in the adult as growth rate declines (see Appendix D for definitions of terms related to bone structure used in this report).

Growth-layer-group counts were carried out on the central area of the dome portion of the periotic bone in my study of manatee age determination. Growth layers were clearly defined in this region, and counts were found to be most consistent with known age or tetracycline calibration relative to other bones examined (see Results). Additionally, Haversian systems were less concentrated and resorption appeared to be less accentuated on the central area of the dome. Age-class estimation using microscopic sections of the periotic dome started with identifying two types of reticular tissue and the subsequent change in structure to compact bone, as noted in the examinations of younger specimens (see Results and Fig. 2.1). The next step was to find the region considered to mark approximately one year of bone growth (see Results and Fig. 2.1). This region was variably characterized by a lighter-stained or unstained thin band ("white rim"), sometimes with traces of an

adhesion line, or in some cases no thin band. In all cases canals in this region changed from more lengthy and horizontally packed in appearance to more shortened and irregularly packed. All other adhesion lines distal to this region were counted as subsequent years. Counts of incomplete, faint traces of lines or individual arms of bifurcated ("double layer" or "split") lines were omitted, because the significance of these patterns has not yet been clearly determined. In my counts of growth-layer groups, the first age class (AC) is defined as age class 0. This age class includes manatees estimated to be less than 1 year of age, including neonates.

Sections were read in 5 independent instances. Microscope filters were used to enhance contrast in sections with less than optimum staining. Adhesion lines were followed as far as possible in resorptive bone and matched to lines on opposite sides of intruding Haversian systems to obtain minimum counts. Photographic montages were employed in instances where simultaneous viewing of several fields was required to accurately count and match growth layers.

Resolution of microstructure of layered hard tissues of dolphins has been improved with the examination of thin ground sections under a polarized light microscope (Myrick, 1980). The principle behind the technique is that the irregular orientation of crystals of hydroxyapatite between layers can be resolved as different colors or shades under

the light-polarizing and phase-contrast capabilities of the microscope. To compare this process with stained preparations, six pieces of earbones and ribs of different specimens were processed at the Geology Department of the University of Florida. Sections were ground to a thickness of 40 μm and glued to a petrographic slide. Subsequently they were examined under a petrographic microscope. Examination of sections under polarized light was not encouraging. In each of the 6 slides examined it was possible to observe microscopic detail such as fracture lines, but growth-layer groups were not distinguishable. This technique was not pursued further because it did not represent an improvement over the decalcification and staining process.

Variability in Counts of Growth-Layer Groups

A sample of histological sections of earbones from 1,078 carcasses was read 5 times in a series of blind replicates, with the time span between each reading ranging from two days to several months. If three out of five readings were the same, the resulting value was used as the age estimate; otherwise an average was taken. This sample did not include those earbones with "best estimates" (AC ~), because of the greater subjectivity involved in estimating age of the latter. Variance was calculated for the five readings of each individual manatee, and the means of the

variances for each age class were computed and plotted against age class.

Determining Effects of Resorption and Seasonality on
Characteristics of Growth-Layer Groups

The amount of resorption observed microscopically in manatee bones was characterized as none, light, moderate, or heavy. Bones undergoing light resorption and remodeling had a low number of Haversian systems. Secondary osteons were found scattered widely in the bony tissue and their presence did not affect growth-layer-group counts. Under moderate resorption, small aggregations of Haversian systems were seen sporadically. Closer attention was required to obtain a reading, and the researcher had to move the stage around these aggregations to count layers. Heavy resorption was characterized by large areas of bone covered by Haversian systems. Counting the lines was difficult, and many of them had seemingly disappeared due to remodeling. The bone looked smooth and devoid of adhesion lines over some portions. More recently deposited Haversian systems obliterated earlier ones in a dense network of secondary osteons. Nevertheless, using counts of existing growth-layer groups together with information on size and other data from the salvaged carcass allows assignment of resorptive adults to approximate age categories when absolute age assignment is not possible.

In cases which were undergoing moderate or heavy resorption, a "best estimate" approach was taken, by examining the structure of the bone, the extent and degree of resorption, and the distances between visible layers. First of all, the "first-year zone" (represented by a change in the structure of the bone) was identified or, if that was not visible, the first adhesion line. Interrupted lines were followed along the microscopic slide whenever possible. When large areas were affected by resorption, the number of lines missed was estimated on basis of the average distance between growth-layer groups, taking into consideration that the first three to six GLG are wider than the subsequent ones. Minimum and maximum possible ages were assigned and the midpoint was used as the estimate of age. As with the other specimens, five readings were taken and the average used as the "approximate age" (~AC).

The relationship of resorption to manatee body length, age class, and sex was examined by logistic regression analysis (Harrell and Lee, 1985). This technique uses maximum likelihood estimation to model a nominal, binary, or ordinal dependent variable as a linear function of both continuous and categorical predictors. A SAS supplemental procedure, LOGISTIC (SAS Institute Inc., 1990), was used to sequentially add terms for length, age class, and sex (0 = female, 1 = male) to logistic regression models that included the ordered resorption categories as the dependent

variable. At each step, maximum likelihood estimates of regression coefficients were calculated, including standard errors and chi-square test statistics for association, improvement of fit, and lack of fit. Hypotheses that resorption was the same for males and females, after adjusting for differences in length and age were tested. In addition, a test was performed for a regression relationship of resorption with age class and length adjusted for sex.

Regression models that included age class as a predictor were restricted to animals where resorption did not interfere with age determination. Consequently, the sample size for such models was reduced because most cases of heavy resorption (all but 10) were excluded.

In a study conducted on dugongs, relative widths of broad zones within outermost growth-layer groups in dentine were compared with penultimate layers and classified by month of carcass recovery (Marsh, 1980). This tabulation showed that growth was slow in winter months. To better determine if layer formation is based on environmental seasonality or is purely endogenous, the width of the outer, most recently formed growth-layer group in known-age Florida manatees was expressed as a percentage of the width of the immediately inward growth-layer group in a similar way as was done by Marsh (1980). If each complete growth-layer group corresponds to 1 year, the thickness of an incomplete growth-layer group should be proportional to the number of

months elapsed since the beginning of its deposition, and the time of line or band deposition could be backtracked. A second approach used was to document, for each individual examined, if the broad band or the adhesion line of the growth-layer group was undergoing deposition at the time of death. Cases where the growth layers were very narrow, or the periosteal layer was overdecalcified (viz., early specimens, kept dry for several years), were excluded from the analysis. A third approach was to look at earbones of small calves below 220 cm total length (which is approximately the length of AC 1 calves) that had been assigned to AC 1. In cases where the "first-year zone" was visible, but little additional bone tissue had been deposited, the last deposition was considered a "line"; in sections where a broad band of tissue followed the first-year zone, deposition at time of death was called a "band."

Correlations of the appearance, width, and other characteristics of layers with known life-history events of well-studied individual Florida manatees were also done. All sections were carefully examined for a regular change in the structure or width of growth-layer groups, and specifically for special marks in the bone of known-age females that might represent known life-history events.

Results

Presence of Growth-Layer Groups in Different Bones

A small series of different bones was examined in several individual manatees (Fig. 2.2, Tables 2.3 and 2.4). Appendix D provides more detailed descriptions of the nature of each type of bone in relation to its potential utility for manatee age determination. The presence of growth-layer groups was most consistent in the dome portion of the periotic.

Layers were also clearly defined in the stapes and tympanic bone. Although the stapes were more easily processed and did not seem to become as brittle over time, numbers of layers in the stapes did not consistently correspond to the number of layers in the dome portion of the periotic bone and were generally fewer (Tables 2.3 and 2.4). Tetracycline labels were never identified in the stapes ($n = 5$). Growth-layer groups in tympanic bones of young animals (up to age class 7) were consistent with counts in the dome area. Vascular primary tissue was mostly absent, or restricted to a small central area. Tympanic bones may be used in young individuals to corroborate the counts in cases where layers are not clearly defined in the periotic dome.

Ribs, mandibles, humeri, radii, ulnae, pelvic bones, phalanges, carpal bones, and hyoid bones did not reliably

show consistent patterns of layering relative to the periotic dome or to either known age or elapsed time since tetracycline administration (Tables 2.3 and 2.4; also see Appendix D, and below).

Growth-layer groups in the periotic dome

Although all the portions of the tympano-periotic bone sectioned (tympanic, dome, and mastoid; Fig. 2.3) presented layering, the number of layers was not consistent among all three (Tables 2.3 and 2.4). The highest number of layers, corresponding most closely to actual age of known-age individuals, was generally found in the dome portion of the periotic bone.

Under microscopic examination, the innermost area of the periotic dome consisted of a primary reticular vascular tissue, where primary osteons (vascular canals not surrounded by Haversian lamellae; see Appendix D for definitions of terms related to bone structure) formed an unorganized, irregular reticulum. The primary osteons had 1 to 3 lamellae and rounded osteocytes. This primary reticular area was observed on the dome and periotic portions, and was followed by a wide band of secondary reticular vascular tissue. These two types of bone were the only kind present in periotic domes of a fetus (MNW-8906F), an 8-day-old captive-born animal (MNW-8907, "Corky"), and two young calves (MSE-8915 and SWFTm-8912B, "Moss"; Fig. 2.4).

The periostic dome exhibited a wide band (noticeable by a change in color and appearance, Fig. 2.1) of transition from reticular bone with circular lacunae to organized lamellar bone with flattened lacunae distal to the secondary vascular tissue, in animals into their first year of age. This band was usually darkly stained. As a rule there was no obvious line demarcating the outer reticular bone from the inner edge of the compact lamellar tissue. The distal edge of this darker band of compact lamellar tissue seems to represent a region marking about one year's growth of bone. Canals in this region shorten in length and become more irregularly packed. A lighter-stained or unstained band ("white rim"), sometimes with traces of an adhesion line, may occur in this region. Subsequent adhesion lines were conspicuously darkly stained, with periosteal layers approximately parallel to the external curvature of the bone (Fig. 2.1).

In some cases growth-layer groups in the periostic bone were variable in definition, with irregular and tortuous deposition. Bifurcated layers were observed (Fig. 2.5B, C). Spacing between layers was not equal, but overall there was a tendency for layers to be closer together as they approached the periosteal edge (Fig. 2.5). Growth-layer groups are more easily discerned in the central portion of the microscopic section of the periostic dome. For that reason, and for purposes of consistency, examination of the

dome for age estimation and measurements of layers were performed in the central area of the section.

Time Calibration of Growth-Layer Groups in Earbones

Growth-layer patterns in neonates

In young animals the periotic dome was primarily composed of reticular vascular tissue. A small fetus (KDL-8738), a near-term fetus (MNW-8906F; Fig. 2.4) as well as two animals recovered dead near birth (MSE-8914 and MSE-8915; Fig. 2.6) presented both primary and secondary vascular tissue. The primary vascular tissue was characterized by a porous appearance, with irregularly spaced vascular canals and osteocytes. The secondary vascular tissue was more dense and contained fewer pores. Animals that were particularly small, presumably very young calves (e.g., UF 13889, M-420, UF 14207 and SWFTm-8912B, "Moss") had variable amounts of dense compact bone in addition to the primary and secondary reticular tissue (Fig. 2.7). This was also the pattern on the bone of the captive-born individual that lived for 8 days (MNW-8907, "Corky"). Osteocytes were arranged in a more orderly fashion in the compact bone. Vascular canals oriented primarily horizontally, giving the bone tissue a more regular and compact appearance.

Growth layers in known-age manatees

MJAV-8921. This manatee (BS-101, "Lester") was first seen at Blue Spring as a very small nursing calf in early

November 1988, probably within a few weeks of birth. It was recovered dead on 20 October 1989 at a total length of 224 cm. Age was approximately 1 year.

In addition to the early reticular material, the dome presented a broad band of compact lamellar bone, without lighter-stained bands or adhesion lines (Fig. 2.8). No subsequent adhesion lines had formed. This manatee was classified as age class 1 (between 1 and 2 years of age) on the basis of these microscopic characteristics of the periotic dome.

SWFTm-9002B. This Blue Spring female ("Liza", BS-87) was born after the 1985-86 winter season, and was first observed as a nursing calf with her mother in early winter 1986-87 (the mother was pregnant without a nursing calf the previous winter). Weaning of SWFTm-9002B occurred prior to the winter of 1987-88. During winter 1989-90 she was observed suffering from trauma and rescued, but she died in January 1990. This animal was, therefore, greater than three but less than four years of age. The carcass measured 266 cm total length.

Histological sections of the periotic dome were characterized by inner layers of primary and secondary vascular tissue, followed by the broad, dark, compact-bone layer and a change in structure and orientation of vascular canals typical of the region marking the end of the first year (Fig. 2.9). Two thin adhesion lines were present

distal to this region, marking the end of the second and third years. The first adhesion line was discontinuous, with greater representation at the center and at the lateral faces, where darker staining made it more conspicuous. The adhesion line marking the third year was continuous and very close to the edge of the bone. Based on the change in structure marking the end of the first year plus two adhesion lines in the periotic dome, this manatee would be assigned to age class 3 (between 3 and 4 years of age), which corresponds to known age. Two adhesion lines were also identified in the stapes, tympanic bone, and periotic portion of the periotic bone.

UF 20774. Rescued from Homossassa Springs on August 13, 1980 at a total body length of 122 cm, this orphaned neonate (M-83-01, SWFTm-8021B, "Violet") was brought to Sea World for rehabilitation. In December 1982 she was transferred to Long Key, Florida, where she died in early January 1983 at a length of 186 cm. Age was therefore approximately 2.5 to 3.0 years. Only the tympanic bone of the right tympano-periotic complex was available for examination. It had been preserved in a mixture of glycerin and alcohol. The transition between the secondary reticular vascular tissue to lamellar bone was quite evident. After the transition, 3 complete growth-layer groups were seen ending in a thin adhesion line (Fig. 2.10). Based on

layering in the tympanic bone (not the periotic dome) she was assigned to age class 3 (between 3 and 4 years of age).

This animal did not receive tetracycline. The count based on the tympanic bone was not reliable in older animals (see SWFTm-8646B and M-229) but may be a good indicator in young animals (Table 2.3). There is no clear explanation for the discrepancy of about one year between this age-class estimate and the estimate of actual age based on the history and size of this animal at rescue (but see Seasonality in depositional pattern). However, examination of growth-layer groups was restricted to the tympanic bone, and it is possible that a different age estimate may have been obtained had the periotic dome been available for examination. In addition, some aspects of growth may have been abnormal in this individual. It was a runt, based on its total body length at death (186 cm) in comparison with wild manatees of similar approximate age measured at Blue Spring (Fig. 2.11).

KDL-8827. This manatee was a Blue Spring female (BS-36, "Felicity") born on May 12, 1980, as determined by direct observation through radio-telemetry studies of its mother (Bengtson, 1981). Specimen KDL-8827 was found dead of unknown causes seven years and eleven months after birth (April 8, 1988) at 312 cm total body length. This female had successfully given birth at age 5 and was pregnant at death. Although not tetracycline-marked, it was the most

valuable specimen because it was a wild animal of known age and history.

All three sections of the earbone (tympanic, dome and mastoid) exhibited the region marking the first year of growth, with an additional six growth-layer groups with thin adhesion lines. In the periotic dome section (Fig. 2.12), the "white rim" was represented by a thin line not conspicuous throughout the bone. Lines 5 and 6 both presented a bifurcated pattern of double lines. The outermost tissue of the periosteum consisted of a light band and was not a constant feature on the surface. Measurements showed it to be about half ($53.3\text{ }\mu\text{m}$) of the width of the immediately preceding layer ($110.8\text{ }\mu\text{m}$). The animal was judged to be age class 7 (between 7 and 8 years of age) and this estimate agrees closely to true age.

This known-age animal also presented the opportunity to compare growth-layer-group counts of different types of bones and degree of resorption present. Evidence of moderate resorption was present on the periosteal layer of the tympanic bone, around the three innermost growth-layer groups. The radius also contained seven layers, but a heavy amount of resorption was apparent. Other bones examined (carpal and hyoid bones) yielded lower counts and showed conspicuous resorption (Table 2.3).

Growth layers in tetracycline-marked and known minimum-age manatees

UF 23993. The birth date of this male is unknown, but he was first identified at Blue Spring (BS-47, "Walter") in November 1981. At that time he was small (total body length 220 cm), within the size range of recently weaned manatees (O'Shea and Reep, 1990). He was therefore assumed by field workers to be greater than 1 but less than 2 years old, and was presumed to be born during 1980. On February 4, 1983 he was captured at Blue Spring State Park (245 cm total body length at capture) and injected with 9,000 mg of tetracycline. On May 2, 1984, 15 months later, his decomposing carcass was recovered following death from a boat collision. The carcass was 265 cm total body length.

This animal's periosteal dome section presented the early reticular tissue followed by a broad, darkly stained band of dense compact bone ending at a "white rim" marking the end of the first year. This was followed outwardly by two complete growth-layer groups. A third growth-layer group was incomplete, with a narrow band of tissue following the second adhesion line (Fig. 2.13). Based on the assumption that a growth-layer group represents an annual increment, UF 23993 was judged to be at least three but less than four years old, or age class 3. This age estimate is compatible with the known history of a probable birth during 1980 and death during late April or early May 1984.

Additionally, this animal was injected with tetracycline 15 months prior to death. Tetracycline was incorporated into that year's growth layer and was followed the next year by another growth-layer group including an adhesion line, visible in the stained section. Additional tissue corresponding to formation of a new growth-layer group also was present. Tetracycline appears as a thin and sometimes faint, but quite distinctive, line nearly throughout the dome section and in part of the tympanic bone. The distance between the label and the edge in both bones corresponds to the location of the second outermost layer in the stained sections. Distances varied between 100 and 398 μm , depending on the depth of the section in each bone, and on the area measured in each section. Portions of tetracycline label varied from 0.15 to 9.05 mm in length. The tetracycline label also confirms that a growth-layer group corresponds to an annual period in the periotic dome. Tetracycline was also present as thin discontinuous lines in the rib and ulna, and was incorporated into plexiform bone and Haversian systems of the mandible.

SWFTm-8321B. This was a small female (231 cm, corresponding to age class 1 or 2) when rescued on August 19, 1983, from entanglement in a crab-trap line. Her left flipper was severed by the nylon line and had to be amputated, hence her name, "Lefty". She received 6,900 mg of tetracycline on November 7, 1984. On September 25, 1992,

at 316 cm total length and approximately 770 kg of weight, "Lefty" died due to reproductive complications 7 hours after giving birth in captivity.

Examination of Lefty's earbone under ultraviolet light did not disclose a label in the dome portion of the tympano-periotic bone, so true age could not be corroborated. The tetracycline lines present in both the tympanic and mastoid portions were continuous, but thin and faint. The stained section of the dome showed 11 growth-layer groups (Fig. 2.14). The first-year zone was followed by a second faint line (corresponding to GLG 2) without a clear adhesion line. Adhesion line 3 was double, and all the subsequent lines were single. There was a considerable amount of bone tissue deposited after adhesion line 11, and resorption was light. According to growth-layer groups, Lefty would have died between 11 and 12 years of age. If she were between 1 and 3 (based on size at capture), and adding 9 years in captivity, the estimate agrees with true age. Resorption was moderate in both the tympanic and mastoid portions, which presented 11 and 8 growth-layer groups, respectively.

UCF-9131. This male manatee ("Ernie") was rescued from the Indian River and taken to Sea World of Florida (SWFTM-8413B) for rehabilitation on 13 May 1984 at 211 cm total length. He had become entangled in a crab-trap line and suffered severe injury to his left flipper. During his stay at Sea World UCF-9131 was administered tetracycline twice:

6,300 mg on 7 November 1984 and 8,400 mg on 3 February 1988 (81 and 42 months before death). Ernie was fitted with a radio tag and released on 6 June 1991. No signals were picked up on 18 or 19 August, and on 20 August 1991 he was confirmed dead, having drowned in a stormwater drain pipe. The carcass measured 275 cm in length.

The dome portion of UCF-9131's periotic shows 7 complete growth-layer groups, followed by a band of compact bone (Fig. 2.15). Therefore Ernie was estimated to be between 7 and 8 years old at time of death. This matches the records, because he was rescued at 211 cm, or approximately the length of an animal about one year of age. Two additional sections are available: the mastoid section only shows 6 growth-layer groups, but the tympanic has 7 complete GLGs. All three sections end in a band of tissue and show no signs of resorption.

The three sections from the earbone (dome, tympanic, and mastoid portions) show two fluorescent labels. In the dome, the innermost label was 960 μm distal to the periosteal border and the second was 384 μm away, at 576 μm from the outer edge. This corresponds, in the stained sections, to areas deposited prior to the first-year zone (793 μm) and past the second adhesion line (589 μm). The distance from the periosteal edge to the second label encompasses 3 complete and 1 incomplete GLG, in accordance with the 3 years and 6 months since the time of tetracycline

administration. The distance from the periosteal edge to the first label contains 6 complete GLG, parts of the newest-forming GLG, and a portion of bone prior to the white rim. This also corresponds closely to the time since administration (6 years, 9 months).

SWFTm-7918B. This female ("Marina") was rescued as a very small calf on 9 August 1979 and remained in captivity until the time of death (16 December 1991) at 290-cm total length. The length at capture (118 cm) places this individual in the category of a young neonate, so estimated age at death is 12.2 years. During 1981 she received intramuscular injections of tetracycline on two occasions: 9 January (5,200 mg) and 16 July (4,000 mg). This represents the longest labeling record in the sample (i.e., 125 and 131 months prior to death). Marina gave birth to a calf less than a month before death; in the process she lost large amounts of blood, which caused renal failure at death.

The estimate obtained through a growth-layer-group count was of AC 11: Marina would have been between 11 and 12 years of age (Fig. 2.16). A line corresponding to AC 12 (between 12 and 13 years) had not been deposited yet, but the last deposition was represented by a broad band (see Seasonality of depositional pattern). Resorption on the dome was light. The sections of the tympanic and mastoid bones resulted in lower counts: 10 and 8, respectively.

The untreated section of the dome, under ultraviolet light, revealed two very bright, clear tetracycline labels. The innermost label was 1,296 μm inward from the periosteal edge, falling just above the location of the first-year zone in the stained section (1,352 μm). The second label was located 384 μm away from the first, corresponding to an area below the first conspicuous adhesion line (i.e., GLG 2) at 891 μm from the periosteal edge of the stained section. This agrees with the history of injections during the first months of captivity.

Double *in vivo* labelling with tetracycline helps to determine true rates of accretion of bone (Harris et al., 1962). Marina and Ernie further confirm the earlier finding of one GLG per year. Marina's case also shows that tetracycline marks persist even 10 years after administration. Slight discrepancies in measurements are likely to be due to measuring layers in two different sections, not necessarily adjacent, and also perhaps not exactly at the same point on both sections. The fact that the first-year zone is a band rather than a well-defined line also reduces precision.

SWFTm-9215B. This large female (350 cm, 1,216 kg) was first sighted at Blue Spring (BS-05, "Phoebe") as an adult accompanied by a calf in 1970. Phoebe became a resident of the run and produced at least five calves since she was first recorded, one of which was KDL-8727 (Felicity).

SWFTm-9215B was lactating when she died, in August 1992, as a result of complications during late-term pregnancy. If the calf accompanying her in 1970 was her first, and she gave birth at age 5 (see Chapter 3) she should have been at least 27 years old.

Phoebe's earbone was undergoing heavy resorption, and only an approximate age could be obtained (Fig. 2.17). According to the readings, the best estimate was of age class 29 (AC ~29). This agrees closely to the estimated minimum age from life history information. Both the tympanic and the mastoid portions presented a very reduced number of growth-layer groups (5 and 7, respectively) due to resorption.

MNE-9102. "Sweetgums" was a female who was first seen at Blue Spring in 1974, as an adult. She returned to the refuge every winter from 1978 on (BS-17). On 4 February 1983, she received 16,400 mg of oxytetracycline at capture for telemetry studies. Eight years and one month later she was fatally hit by a boat. Sweetgums had calved on at least 4 occasions, the last time 1.5 years prior to death. She was 343 cm long and lactating at the time of death (2 March 1991).

Moderate resorption and a pattern of double and closely-appressed growth-layer groups in MNE-9102 only allowed an approximation to the age at death. The average of 5 readings resulted in an estimate of approximately 19

years of age (possible range 17-20). The only apparently double layer was GLG 8 (Fig. 2.18). Resorption was heavy in the tympanic, with a minimum of 10 growth-layer groups, and light on the mastoid, with a reading of 10 growth-layer groups.

The tetracycline label was narrow, dull, and discontinuous but still recognizable under ultraviolet light. Tetracycline was also incorporated in the Haversian systems and in the mastoid portion of the earbone. Independent from the fact that true age was not known and the age estimate was an approximation, the distance between the periosteal edge and the label ($306\text{ }\mu\text{m}$) roughly corresponded to the area between the 7th and 8th adhesion lines inwards from the periosteum in the stained section ($346\text{ }\mu\text{m}$). This corresponds well to the known elapsed time of 8 years and 1 month between tetracycline administration and death.

UF 20773. This male was a Blue Spring winter resident (BS-6, "Gandalf") first observed in 1970. He died in late November 1982 as a result of intussusception of the small intestine caused by ingestion of monofilament fishing line. Twenty-two months prior to death (February 2, 1981) he was captured and administered 21,000 mg of tetracycline. He was a large individual (338 cm long at death) and was estimated to be fully adult when first recorded in winter 1970-71.

A thin section of the periotic dome viewed under ultraviolet light showed a tetracycline label in several small segments, with an average distance of 26.89 μm from the periosteal edge. Analysis of the stained section equivalent to the same area produced an average distance for the outermost layer from the periosteal edge of 12.37 μm , and for the second 25.57 μm . Thus, the two outer broad bands represent approximately 2 years, which corresponds with the 22 months elapsed between tetracycline administration and death. Tetracycline also showed as a discontinuous line in the mastoid portion and in mandible and rib sections. It was also seen in Haversian systems of the tympanic and pelvic bones, mandible and rib.

The "best estimate" approach yielded an AC ~28 or a range 26 to 32 growth-layer groups (Fig. 2.19). The tissue was undergoing heavy resorption, and some previously existing layers may have been obliterated by this process. The distance between double lines in growth-layer groups 12, 16 and 19 was narrower than the distance between those and subsequent growth-layer groups; the lines also followed a split-and-join pattern along the slide. Therefore these double lines were counted as single years. Towards the periosteal edge (growth-layer groups around 28), single lines were counted as annuli due to their continuity throughout the section. This animal would be estimated to be an old adult greater than 28 years of age; an estimate of

true chronological age could not be made because of resorption. This estimate is compatible with its prior history and size.

UF 20758. This female manatee (MSQ-6, "Alexandra") was born in captivity at Miami Seaquarium on August 3, 1980. She received 3,740 mg of oxytetracycline on March 26, 1981. The animal accidentally drowned in its holding tank on 28 August 1982 at 210 cm total body length.

Distinctive adhesion lines were not present on the dome portion of the periotic bone, although two adhesion lines (one of which was bifurcated) appeared in the stapes, and one in the tympanic bone. In the periotic dome, the core showed the characteristics of reticular vascular bone, followed by a wide, dark band of compact lamellar bone. The "white rim" was followed by a wide band of tissue, with more irregularly oriented canals (Fig. 2.20). Based on this structure, UF 20758 was categorized as age class 1 (between 1 and 2 years of age), which was an underestimate of true age (2 years, 25 days).

The untreated slide examined under ultraviolet light showed a strong tetracycline line 1.2 mm inwards from the periosteal edge. Similar lines were also found along sections of the tympanic and mastoid portions (1.49 mm and 0.71 mm from the edge, respectively). The "white rim" area, taken to be a transitional mark at about the one-year period, was distal to the tetracycline line (0.26 mm),

confirming the interpretation of the chronology of deposition of this region (Fig. 2.21). Tetracycline was also identified along the primary canals and resorption front of the rib, in the plexiform bone of the mandible, and in the cochlea.

UF 20757. This subadult-sized (247 cm total body length) female (SWFTm-927B, "Panama") was rescued from entanglement in a crab trap line on September 17, 1979 and transported to Sea World of Florida for rehabilitation. Septicemia around the spine and multiple subcutaneous abscesses demanded therapeutic use of tetracycline. From February 23 to 25, 1980 she received 4,000 mg of oxytetracycline twice daily by intramuscular injection; between February 25 and 28 she was given 10 g twice each day orally; and from March 3 to 6 she was administered 10 g intravenously once each day. The animal died on September 17, 1980. This manatee was 233 cm at death, a reduction in total body length while in captivity because of progressive ankylosis of the spine.

The periotic dome section was composed of primary and secondary vascular tissue bordered by a lightly-colored narrow band. This band also marked a region where tissue structure changed from lamellar to more compact bone with fewer vascular canals, which was described as a region marking the first year of bone growth. Three subsequent growth-layer groups followed (Fig. 2.22), with a small

amount of tissue present distal to the last adhesion line. Based on these criteria, this manatee was assigned to age class 4 (between 4 and 5 years of age). This classification is consistent with the range of ages associated with body lengths of wild manatees similar to that of UF 20757 at capture (Fig. 2.11) and the additional year in captivity.

Repeated therapeutic administration of tetracycline resulted in distinct labeling of some bones. Only a very short amount of label ($48\text{ }\mu\text{m}$) was seen on the dome portion of the periotic bone, and this was very close to the edge ($11\text{ }\mu\text{m}$). The distance between the outermost layer (corresponding to the end of year 4) and the edge on the stained section was $38.8\text{ }\mu\text{m}$. Wide bands of tetracycline were present on the mandible (both longitudinal and transverse sections). Notwithstanding the dosage, tetracycline was not evident in the ribs; however, this animal showed severe ankylosis, with deformed thoracic vertebrae, and perhaps this condition affected presence of the label.

M-453. This female (BC-69) was first identified as an independent subadult-sized animal in field studies in the Banana River, Brevard County, Florida, in winter 1978-79. Researchers estimated its body length on three dates during this winter as 6-7 feet (183-213 cm), 8 feet (243 cm), and 8 feet (243 cm). These estimated body lengths are within the size range of manatees approximately 1-3 years of age (Fig.

2.11). The 292-cm carcass was recovered in May 1985, approximately 6.5 years after the first sighting; thus, the animal probably was between 7.5 and 9.5 years old at death.

The periotic dome was characterized by a transitional area corresponding to the end of the first year, and six additional complete growth-layer groups (Fig. 2.23). This configuration resulted in its categorization as an age class 7 manatee (between seven and eight years of age). This estimate conforms with the elapsed time since the initial sighting and its estimated young age at that time, as well as with lengths of known 7-8 year-old animals (Fig. 2.11).

UF 24971. This large (387 cm) Crystal River female (CR-39, "Josephine") was classified as an adult when first identified in winter 1977-78. She returned with a calf in winter 1978-79 and was judged to be pregnant in 1980-81. Her badly decomposed carcass was found in 1984 (M-382).

Some of the early adhesion lines were faint, and the earbone was undergoing moderate resorption, but all the lines seemed visible. Fifteen growth-layer groups were seen in addition to the change corresponding to one year's growth. Adhesion lines included double, multiple and faint lines (Fig. 2.24). According to these characteristics, she was classified as age class 16 (between 16 and 17 years of age).

SWFTm-8646B. This large Blue Spring female (BS-9, "Emma") was captured for radio-telemetry studies on 19

January 1981, when she was administered 22,800 mg of tetracycline. She was found dead 66 months later (August 5, 1986). Length at death was recorded at 300 cm, but the carcass was in advanced stages of decomposition, perhaps accounting in part for a discrepancy with a previous length measurement of 317 cm taken at capture in 1980. She was recorded as an adult female with a calf in winter 1970-71, the first year of record-keeping at Blue Spring. Because age at first reproduction in manatees can be expected to be approximately 4 (see Chapter 3), this manatee should have been no less than 20 years old at her death. If she had her first birth at a greater age and the calf she nursed in 1970-71 was not her first, then she could be considerably older than this minimum.

A range of 15 to 24 GLG, with a best estimate of 19 (AC ~19) was obtained for SWFTm-8646B's dome area of the periotic. Consistent with the approach used in interpretation of closely appressed adhesion lines in this study (e.g. KDL-8827 and UF 20773 described above), double (2, 4) or multiple (6, 7, 9) adhesion lines were counted as one growth-layer group each (Fig. 2.25A, B). The number of complete growth-layer groups was 9 in the tympanic bone and 12 in the mastoid. Tetracycline was observed as a few short labels (varying from 185 to 707 μ m in length) very close to the edge. The average distance between the tetracycline label and the outer edge of the dome (66 μ m), was

considerably smaller than the distance from the edge to the 4th (135 μm) and 5th (186 μm) outermost adhesion lines. If the fluorescence matched the stained section, the label should have fallen in that vicinity. Indeed, in one of the "thick" sections where the adhesion lines were distinguished, the label seemed to be located on the outermost edge of the bone. Another section shows a more diffuse fluorescence at one edge, encompassing a broad area, rather than a line. Fluorescence was also observed on the tympanic bone, carpal bone, radius, ulna, humerus, rib and mandible sections, but not on the mastoid portion of the tympano-periotic complex, in the phalanges, or in the stapes.

The reduced number of layers relative to minimum age can be accounted for by the presence of heavy resorption. Some of the layers were obliterated and not possible to count, so age estimation was based on the "best estimate" approach. Resorption and perhaps lack of growth or changes in growth patterns may also account for the presence of tetracycline label in regions inconsistent with growth-layer-group counts interpreted as annuli.

Growth-layer groups in other sirenians

Eighteen specimens (17 from Puerto Rico and 1 from Belize) of the subspecies *Trichechus manatus manatus* were available (Table 2.2). Body lengths ranged from 119 to 335 cm. The depositional pattern conformed with the pattern

observed and described for Florida manatees, even though *T. m. manatus* lives in a more tropical habitat, where cool weather occurs much less often than in Florida. A typical section consisted of reticular tissue, followed by lamellar tissue bordered by a poorly-stained band, as described under Growth-layer groups in the periotic dome. A pattern of bands and adhesion lines followed, with some cases of double and split lines, especially in larger specimens. Similar to the pattern seen in Florida manatees, resorption became more intense as the animals aged and affected readings of animals greater than AC 20. Various body-length values may correspond to different age classes (Table 2.2).

On the other extreme, the Steller's sea cow (Dugongidae: *Hydrodamalis gigas*), a species of sirenian that was exterminated by hunting by the mid 1700's, inhabited the Bering Sea in the Arctic, and was the only sirenian to occupy cold waters (Forsten and Youngman, 1982). Analyses of an earbone (UC Berkeley 23031) demonstrated that the Steller's sea cow depositional pattern was very similar to the general pattern seen in the two species of manatees (Fig. 2.26). Brittleness and antiquity (over 200 years since death) of the bone contributed to difficulty in preparation. The outer layers were damaged by the decalcifying agent, and only a minimum of 16 layers was counted.

Seasonality in the depositional pattern

I did not repeat Marsh's (1980) analyses based on microscopic dentinal layers in tusks because problems were encountered with the use of this method in manatees: (a) the bone layers generally become narrower toward the periosteal edge, with a more dramatic decrease in width occurring anywhere from the 3th to the 8th growth-layer group, (b) in old animals, a difference between the last and the few previous layers is hardly noticeable, and (c) finally, in some cases, the last growth-layer group may be broader than the previous one (Table 2.5). This approach may, therefore, be misleading in Florida manatees, because the pattern of deposition may change with time. For instance, Liza's last deposition was a small band corresponding to 24.5% of the previous growth-layer group, and she died in January; Sweetgums had 73.8% of the width of the last growth-layer group deposited (band) and it was only March when she died. However, she was estimated at AC 19, and all the last layers were closely appressed. Finally, Marina's last growth-layer group was larger than the previous one (Table 2.5).

Examination of the outermost deposit (presumably at time of death) in the periosteum of the dome (band or adhesion line) of all earbones processed showed a slight tendency for lines to occur with greater frequency in winter months. In contrast, most animals estimated at AC 1 and found dead during the winter only had deposited the white

rim, whereas most of those found dead further into the year had more tissue deposited (Fig. 2.27). This is an indication that at least the first-year zone is formed during the first year of life.

Some studies have shown correlations between life history parameters and the depositional pattern in teeth. For instance, Bengtson (1988) found marks of suckling and fasting events in Antarctic fur seal pups (*Arctocephalus gazella*); Klevezal' and Myrick (1984) described deep-stained layers in *Stenella* correlated with parturition events; and Hohn (1991) identified a "transition zone" in bottlenose dolphins (*Tursiops truncatus*) that compares well with information on attainment of sexual maturation. A change in the structure of the bone after the first-year zone, that might represent attainment of sexual maturity, could not be identified in manatees, and no correlation was found between lactation and gestation episodes in females of known reproductive history.

Variability in growth-layer-group counts

Best precision was obtained in the lower range of ages where the annual growth-layer groups are usually wide and better defined and where resorption is minimal. Variability in the counts of growth-layer groups increased with age (Fig. 2.28) as demonstrated by the range of variance values in age classes greater than 10.

Growth-Layer Groups in Relation to Size, Sex, and Resorption

Findings of heavy resorption accompanied by growth-layer-group counts lower than known minimum age in SWFTM-8646B suggest that resorption decreases the reliability of growth-layer-group counts as a method of absolute age determination in adult manatees. To investigate this problem further, data were analyzed for 1,212 manatees (16 known-age and 1,196 additional specimens) classified on the basis of resorption category, age class, total body length and sex (Tables 2.1, 2.2 and Appendix A). In most cases, resorption was not observed or was classified as light in either sex until total body lengths of 300 cm or more were achieved (Figs. 2.29). Resorption also was not observed or was classified as light in age classes up to about 15 years (Fig. 2.30).

Stepwise logistic regression was used to examine the relationship of resorption to age, length, and sex. Values of length and age were log-transformed to correct for curvilinearity detected in arithmetic scale, and sex was recoded to a numeric "dummy" variable (0 = female, 1 = male). Resorption, which consisted of four ordinal levels (0 - 3), was regressed against the predictors in a stepwise sequence. Cases where resorption interfered with age determination were excluded from the analysis. Analyzed separately, both age and length were highly significant ($P < 0.0001$) when added to models after sex, indicating that

older and larger animals were more likely to show the highest resorption levels. Adding length to a model that already contained sex and age, however, did not significantly improve the fit ($P = 0.0941$) (Table 2.6). There was also a highly significant sex effect ($P < 0.0001$), because females were more likely to have higher resorption levels after adjusting for age and length differences (Table 2.6). There was no evidence of interactions involving sex with age or length ($P > 0.45$).

The lack of reliability of bone growth-layer-group counts for absolute age determination in manatees in the presence of significant resorption also was evident from other case examples listed on Appendix A. Manatee UF 20608 was a very large (344 cm, 998 kg at capture) adult female rescued in September 1977 and held in captivity until death in February 1984. Complete growth-layer-group counts were difficult because of heavy resorption, but a minimum of 15 were present. Clearly this individual was older than 15 years because it was very large at the time of capture, reproductively inactive (and perhaps reproductively senescent), and at necropsy showed arteriosclerosis and other old-age characteristics rarely seen among salvaged specimens. The best estimate (using criteria described in Determining Effects of Resorption...) for the individual was 22. Similarly, UF 24989 was an animal recognizable from Crystal River field studies as an adult for 6 years (Rathbun

et al., 1990). This female had a total body length of 375 cm, and at 1,620 kg was the most massive manatee ever recorded. Despite these indications of extended age, due to heavy resorption only 14 growth-layer groups were visible. The best estimate for age in this individual was of AC ~28, based on the average of 5 readings using the "best estimate" approach.

To verify general relationships between body length and age, I compared age-class assignments based on growth-layer-group counts from dead specimens with the range of total-length measurements determined from a separate group of living, known-age wild manatees from Blue Spring (Fig. 2.11). Field data for this comparison were from wild manatees measured each year up to age class 9, with body lengths approaching a maximum of 300 cm (O'Shea and Reep, 1990). Growth-layer-group counts for the comparison were based on specimens from Appendix A that fall within the range of ages of live manatees measured at Blue Spring. This analysis supported the above results, and the finding that growth-layer-group counts in the periotic dome are accurate estimates of true age for manatees up to at least 300 cm total body length and at least through the first 10 years of life.

Maximum Age-Class Assignments and Longevity Estimates

Despite resorption-based interference in age estimation through growth-layer-group counts, some individuals had high counts (Appendix A). For example, 59 growth-layer groups were estimated in UF 15115, a 362-cm female (Fig. 2.31); ~37 in M-229, a 309-cm male; and several large animals over 300 cm had growth-layer-group counts of over 20 (Fig. 2.32). Such growth-layer-group counts are not inconsistent with other knowledge of manatee longevity from field studies and in captivity (Table 2.7), and with 70-year longevity estimates for the dugong (Marsh, 1992).

Discussion and Conclusions

Results of this study indicate that microscopic examination of the dome portion of the periotic bone of Florida manatees can provide a means to estimate age. An age-estimation technique based on counts of growth-layer groups corresponding to annuli in this bone seems especially accurate for individuals of ages ranging up to about 10-15 years and body lengths up to about 300 cm. This generalization was verified by case histories of a total of 16 individual manatees that were either known-age, had minimum ages established by other criteria, or had tetracycline markers in bone. Case studies of three Blue Spring manatees ranging from about 1 to nearly 8 years of age showed close agreement between estimated age and true

age; location of fluorescent marks and subsequent layering patterns demonstrated that layers were annual and corresponded well to elapsed time since tetracycline administration in three Blue Spring animals and three captive manatees. Fluorescence marks and growth-layer counts did not correspond with known minimum age or elapsed time since tetracycline administration for one large adult female from Blue Spring. However, this specimen was characterized by heavy bone resorption. Analysis of other specimens also showed that resorption was likely to interfere with age estimation for larger, older manatees.

For mammals in general, deposition of thin adhesion lines corresponds to a regular, seasonal slowing of growth in winter months (Klevezal', 1980; Klevezal' and Kleinenberg, 1969). However, exceptions to this generalization also occur. For example, in some sciurids adhesion lines seem to be deposited at the end of spring (Castagnet, 1980), and in elephant seals (*Mirounga leonina*) and harp seals (*Pagophilus groenlandicus*) the thickest growth layers, corresponding to rapid growth, are deposited during the winter feeding period (Bowen et al., 1983; Laws, 1952). Absence of strong demarcations in the first bone-growth layers of mammals are considered to be due to lack of pronounced fluctuations in growth rates during early life (Klevezal' and Kleinenberg, 1969). Seasonal changes in environmental conditions affecting growth and nutrition

beyond these early years have been assumed to result in differential widths of growth layers in dugong tusks (Marsh, 1980). Hypocalcified dentinal layers in growth-layer groups of dusky dolphins (*Lagenorhynchus obscurus*) were thought to reflect environmental changes under El Niño conditions (Manzanilla, 1989). Other studies, however, indicate that formation of growth-layer groups in marine mammals may be largely based on endogenous rhythms (Hohn et al., 1989).

Examination of manatees younger than one year revealed that changes in cell types, packing, and shape and orientation of vascular canals are keys to assignment of age categories based on early growth. Although neonatal lines representing the time at birth have been identified in teeth of odontocetes and dugongs, a neonatal line is not present in domes of manatees. Neonatal lines usually consist of a thin translucent or opaque layer and are thought to occur due to disturbances in the nutrition of the animal in the immediate postpartum period (Perrin and Myrick, 1980). The identification of a neonatal line is usually based on specimens caught soon after birth. My sample contained several large fetuses and small calves, but a neonatal line could not be detected in any of those specimens.

Unlike some species of mammals, the first year of life in manatees is marked by a zone of osteological change with a variable appearance, but it is not typically or consistently characterized by an adhesion line. This first-

year zone is characterized by a change from lengthy and horizontally packed canals to more shortened and irregularly packed canals; the first-year zone may occur with or without the presence of a lightly stained or unstained band ("white rim") or traces of an adhesion line.

The first strong thin adhesion line observed in microscopic sections seems to be formed at the end of the second year, as demonstrated in some of the known-age animals. For example, MJAV-8921, known from the field to be older than 1 but less than 2 years of age, had a first-year zone in the periotic dome, but no adhesion lines. Manatee SWFTm-9002B, known to be between 3 and 4 years of age, had her first dark adhesion line two growth-layer groups inwards from the outer edge of the periotic dome. Similarly, counting of the layers in the dome of SWFTm-8646B (the 7.9-year-old wild female), from the edge of the bone inward, results in the innermost (first) adhesion line corresponding to year 2.

Various hypotheses can be advanced to account for timing of the formation of the first strong adhesion lines in Florida manatees. I was unable to determine clear-cut relationships between widths of deposition zones and various sub-annual indications of periodicity to unequivocally support any one of these hypotheses, and their relative importance at this stage of knowledge is purely speculative. For example, all growth-layer groups may be formed based on

a purely endogenous rhythm. Alternatively, growth-layer groups may be formed endogenously, but the timing of the formation of the first thin adhesion line may be based on other events. The energetic stress of the first winter of life might result in formation of the first thin adhesion line in Florida manatees. Alternatively, growth rate may not be appreciably slowed during winter in calves that are nursing, and the first adhesion line might represent the first winter after weaning. Because weaning occurs at variable ages and calves can be nursed for one winter or two (Rathbun et al., in manuscript), under this hypothesis growth-layer-group counts also could underestimate true age in some cases, but at most by 1 year. At least the first-year zone appeared to be formed during the winter. The occurrence of multiple well-defined growth-layer groups in the ten adult specimens from Puerto Rico, and one adult specimen from Belize (where seasonal climatic changes are more moderate than in Florida), however, indicates that exogenous effects alone do not fully account for adhesion-line formation. Examination of the specimens from Puerto Rico and Belize also suggests that age-determination studies using the periostic dome can eventually be extended to the Antillean subspecies in the tropical range of the West Indian manatee.

Bones other than the periostic dome can be categorized as a) those where periosteal layering is absent or

negligible due to rapid resorption (e.g., pelvic bone, mandible), and b) those whose periosteal layer contains adhesion lines, with the total number not corresponding to age (ribs, forelimb bones, stapes, tympanic bone). Previous investigators have speculated that growth-layer groups in some of these other bones (particularly mandibles and ribs) might have utility in age-determination studies of sirenians (Domning and Myrick, 1980; Marsh, 1980; Mitchell, 1978; Odell, 1977). However, examinations of these bones suggest that they are less useful for age determination than the periotic dome. Mandibles are largely composed of plexiform bone, and counts of layers are inconsistent (usually lower) with known age or with counts in the periotic dome. The mandibular layering pattern, with the presence of osteoclastic resorption, makes the jaw a less-than-ideal bone for age determination in other marine mammals (Brodie, 1969; Hay, 1980; Marsh, 1980; Watts and Gaskin, 1989). In manatees, ribs and long bones also undergo considerable amounts of remodeling, rendering counts of layers unreliable. Phalanges and pelvic bones have large numbers of Haversian systems surrounding a cancellous core. Growth layers at the periotic dome were easier to count, occurred in greatest numbers, and were more consistent with known age. Most growth-layer-group counts in the tympanic bone of manatees up to 7 years of age, however, compare positively with counts in the periotic dome. Tympanic bones,

therefore, may be used to corroborate age estimation when counts in the periosteal dome leave room for doubt, particularly in young animals where the first adhesion line may not be clearly defined.

Drawbacks to the use of growth-layer-group counts in the periosteal dome for age determination are related to the histomorphological pattern of Haversian substitution of bone tissue with increasing age. In the process of remodeling, periosteal bone is replaced by secondary osteons and previously formed layers disappear. Bone is an intensely active tissue that plays a major role in mineral homeostasis, and resorption of bone is a complex phenomenon. Intense Haversian substitution and accumulation of osteons takes place with age in mammals (Currey, 1964; Evans, 1977; Martin and Burr, 1989; Ortner, 1975; Stout and Simmons, 1979). The efficiency of utilization of ingested calcium also decreases with age (either due to decreased resorption from the gastrointestinal tract or poor retention by tissues). Experiments with cattle show that absorption decreases with age even with large amounts of available calcium (Hansard et al., 1954).

In addition to the effects of age, however, abnormal resorption may be triggered by pathologic processes such as hyperparathyroidism, invasion by neoplastic cells, osteomyelitis, mechanical pressure, or chronic dietetic mineral and vitamin imbalance (excess of vitamin D or

insufficient calcium intake) (Hancox, 1972). The amount of bone mineral in the skeleton also depends on the calcium content of the diet as well as age (Bruce and Wiebers, 1970; Gaster et al., 1967; McLean and Urist, 1968; Williams et al., 1964). Bone mineral reserves are reduced during periods of curtailed feeding (Bruce and Wiebers, 1970), and diets poor in calcium, phosphorus, and magnesium may result in lower amounts of these minerals in bones of some domestic animals (Maynard and Loosli, 1969; Underwood, 1981). Trabecular bone contains the most labile fraction, and is rapidly formed and destroyed in birds and mammals with shifts in calcium requirements (Martin and Burr, 1989; Meister, 1956). Enforced immobility, hibernation, and hypothermia also may cause bone demineralization (Bruce and Wiebers, 1969; Haller and Zimny, 1977; Haymovits et al., 1976; Kwiecinski et al., 1987; Steinberg et al., 1981). Reproduction and lactation are calcium-depriving situations regardless of the amount of calcium in the diet, and can impose additional stress on mineralization of bones in females (Atkinson and West, 1970; Goss and Schmidt, 1930; Komárková et al., 1967; Kwiecinski et al., 1987; Pitkin et al., 1979; Rasmussen, 1977; Spray, 1950; Teague, 1988; Toverud and Boass, 1979).

Variability in the amount of resorption seen in bones of different manatees may therefore be expected to be high, allowing more accurate counts of growth-layer groups in some

individuals than in others. In Florida, individual manatees are known to differ in migratory patterns (Rathbun et al. 1990; Reid et al., 1991); they are also known to spend differing amounts of time feeding in areas with markedly different ecological characteristics (such as freshwater habitats where food consists of submerged or natant aquatic plants, tidal marshes where cordgrass is the predominant food, or marine seagrass meadows) (Ledder, 1986; Lefebvre and Powell, 1990; Zoodsma, 1991); they have differing histories of exposure to environmental stresses such as cold temperatures, disease, or food deprivation (Bengtson, 1981; Buergelt and Bonde, 1983; Buergelt et al., 1984; Frye and Herald, 1969; Irvine, 1983; O'Shea et al., 1985); and different histories of pregnancy and lactation exist among females (Bengtson, 1981; Rathbun et al., in manuscript). When adjusted for age, logistic regression revealed that earbones from female manatees are more prone to suffer resorption than earbones from male manatees. This is consistent with a higher demand for calcium during lactation periods.

In the periotic dome of manatees, resorption seems to ensue at a later age and early layers are not as readily obliterated as in other bones. In some cases the vestiges of adhesion lines retained between the osteons allow an approximate count. In other cases, resorption is extensive, the number of layers is reduced, and precise age estimation

is clearly an unrealistic expectation. Older animals also may impose difficulties in counting growth-layer groups because, as calcium deposition decreases with age, the layers become compressed towards the periosteal edge. [This conforms with a general mammalian pattern representing a reduction in growth with age (Kohn, 1978; Peters, 1983)]. Additionally, older animals pose greater judgmental difficulties related to the counting of incomplete, closely appressed or double lines. This was apparent from some of the specimens [e.g., SWFTm-8646B (Fig. 2.25) and UF 20773 (Fig. 2.19)], where counting of each line could have resulted in higher age estimates than those obtained. It is judged here best to be conservative and omit counts of some secondary adhesion lines as annuli because some specimens (e.g., KDL-8827) verify that double lines can occur within years.

All criteria for age determination are subject to a certain amount of error, which may include random variability about some true age as well as bias at some ages due to methodology (Kimura and Lyons, 1990). Methods based on annually-repeating characteristics tend to produce the best results (Caughley, 1977). Different authors have opposite ideas on the degree to which errors in ageing influence the final results of a study (Caughley, 1977; Robinette et al., 1957), but it is clear that errors are inevitable. Therefore it is important to consider potential

errors in estimation when correlating age with growth, reproduction, and mortality. A major source of error in reading growth-layer groups in manatees is the difficulty in distinguishing layers. This difficulty can be due to resorption, to patterns of double or split layers, or to closely-appressed layers. Because of this, a certain amount of subjectivity by the investigator is unavoidable.

Age determination is a statistical process with a characteristic level of variability (Kimura and Lyons, 1990), and the magnitude of error may be estimated with a statistical confidence interval for the conclusions, by analyzing a large sample of known-age animals. When, as in the case of the manatee, such a sample is not available, only a "within-reader" estimate of error for a single reader of a large sample can be provided. Best precision in age estimates was obtained at the lower end of the age scale. This is important for population modeling by removing potential bias in the calculation of age-specific reproductive rates for age classes around the time of sexual maturation (Mansfield, 1991). Factors affecting precision in manatee age estimation include the difficulty in reading some sections due to the complexity of the depositional pattern, the degree of resorption in individual bones, and poor preparation in brittle specimens.

Table 2.1. Known-age, known minimum-age, or tetracycline-marked Florida manatees used for evaluation of bone growth-layer groups as an age-determination technique. See text for further details. See Key to Symbols for explanation of specimen abbreviations.

Identification number	Sex	Length (cm)	Death date	AC	RC	Comments
UF 20774	♀	186	9 Jan 1983	3	N	Rescued 13 Aug 1980 as orphaned neonate. Captive, about 2.5 years at death. Periotic dome not available
UF 20758	♀	210	28 Aug 1982	1	N	Captive-born 3 Aug 1980. Two years old at death, 17 months between injection (26 Mar 1981) and death.
MJAV-8921	♂	224	20 Oct 1989	1	N	Slightly over 1 year of age.
UF 20757	♀	233	17 Sep 1980	4	N	Captive subadult, 7 months between injection (23-28 Feb 1980) and death.
UF 23993	♂	265	2 May 1984	3	N	Subadult, 15 months between injection (4 Feb 1983) and death.
SWFTm9002B	♀	266	30 Jan 1990	3	N	Young nursing calf at first sighting (winter 1986-1987). Died three years later.
UCF-9131	♂	275	20 Aug 1991	7	N	Rescued as a calf, captive for seven years. Tetracycline-injected 80 and 42 months prior to death.

Table 2.1.--continued

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Identification number	Sex	Length (cm)	Death date	AC	RC	Comments
SWFTm7918B	♀	290	16 Dec 1991	11	L	Rescued as a calf 9 Aug 1979. Tetracycline-labeled 125 and 131 months prior to death.
M-453	♀	292	12 May 1985	7	N	Subadult at first sighting (19 Dec 1978); 6.5 years between first record and death.
KDL-8827	♀	312	8 Apr 1988	7	L	Known-age: 7 years and 11 months at death. Born May 1980.
SWFTm8321B	♀	316	25 Sep 1992	11	L	Rescued as a calf 19 Aug 1983. Tetracycline-marked 84 months prior to death.
SWFTm8646B	♀	317	5 Aug 1986	~19	H	Adult, 66 months between injection (19 Jan 1981) and death. At least 21 years old at death.
UF 20773	♂	338	27 Nov 1982	~28	H	22 months between injection (2 Feb 1981) and death.
MNE-9102	♀	343	2 Mar 1991	~19	M	Adult, known since 1974. Ninety-seven months between label (4 Feb 1983) and death.
SWFTm9215B	♀	350	6 Aug 1992	~29	H	Adult, known since 1970. Twenty-two years since first sighting and death.

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Table 2.1.--continued

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Identification number	Sex	Length (cm)	Death date	AC	RC	Comments
UF 24971	♀	387	1 Apr 1984	16	M	Adult, 6.5 years between first sighting (25 Nov 1977) and death.

Abbreviations: AC = age class; RC = resorption category: N = none, L = light, M = moderate, H = heavy.

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Table 2.2. *Trichechus manatus manatus* specimens analyzed in this study for presence of growth-layer groups. All specimens were collected in Puerto Rico, except for SB-Belize-1. See Key to Symbols for explanation of specimen abbreviation.

Identification number	Sex	Length (cm)	Death date	Age class	Resorption category
NM 550416	♂	313.0	Sep 80	27	H
NM 550417	U	335.0	May 81	~26	H
NM 550419	♀	335.0	Jan 82	15	M
UF 18791	U	NA	Feb 70	2	N
M-420	♀	119.5	Feb 85	0	N
M-427	♂	297.0	Feb 85	26	M
NEPST019	♀	300.0	Aug 88	~24	H
NEPST106	♀	208.0	Jun 89	1	N
NEPST112	♀	329.0	Jan 90	12	M
NEPST113	♀	174.0	Jan 90	2	N
NEPST124	♀	206.0	Oct 90	1	N
NEPST142	♀	278.0	Jan 91	~25	H
NEPST149	♀	253.0	Feb 91	9	L
NEPST150	♀	210.0	Apr 91	1	N
NEPST163	♂	138.0	May 91	0	N
NEPST164	♀	300.0	May 91	~25	M
NEPST172	U	NA	Sep 91	10	L
SBBELIZE1	U	NA	NA NA	15	M

Abbreviations: N = none; L = light; M = moderate; H = heavy; NA = not available; U = undetermined.

Table 2.3. Types of bones examined for growth-layer groups and age-class estimates obtained from each, in the sample used for validation. Samples characterized by heavy resorption are indicated with an asterisk (*). See text and Table 2.1 for the basis of actual age assignments. Approximate known-age is symbolized by (~). See key to Symbols for explanation of specimen abbreviations.

Identification number	Age	St	Ty	Do	Ma	Rb	Hm	Rd	Ul	Px	Cp	Pv	Md	Hy
UF 20758	2.0	0	1	1	2	4							0	
UF 20774	~2.5		3			4								
UF 23993	~3.5	4	3	3	1	4							0	
SWFTm-9002B	~3.5	2	2	3	2									
UF 20757	3-4	2	3	4		3							0	
UCF-9131	>7		7	7	6									
KDL-8827	7.9		7	7	7			7			5			5
SWFTm-8312B	>9.0		11	11	8									
SWFTm-7918B	12		10	11	8									
MNE-9102	>17		10	~19	10									
SWFTm-9215B	>27		5	~29	7									
SWFTm-8646B	>21	1	9*	~18	12*	12	11	5	5	2	5		0	
UF 20773	old	>3*	>2*	~27	>8*	*15						4	0	

Abbreviations: st = stapes; ty = tympanic; do = dome; ma = mastoid; rb = rib; hm = humerus; rd = radius; ul = ulna; px = phalanx; cp = carpal; pv = pelvic; md = mandible; hy = hyoid

Table 2.4. Age-class estimates obtained from growth-layer-group counts in 4 areas of the tympano-periotic complex, for specimens of unknown age. Ages assigned based on "best estimate" approach are symbolized by (-). See Key to Symbols for explanation of specimen abbreviations and Appendix B for other identification.

Identification number	Dome	Stapes	Tympanic	Mastoid
CM 77801			5	
LACM 72233	3		1	
MSB 50451			0	
MSB 50452			0	
MSB 50453			0	0
MSB 50458			0	
MSB 50462			0	0
UF 13918			4	
UF 15115	~59	1		
UF 20592	0	0		
UF 20597	0	0		
UF 24977	8	2		
KDL-8715			0	
KDL-8722	1		1	1
KDL-8802	2	1		
KDL-8805	1	0		
KDL-8830	1	1		
KDL-8837	0	0		
KDL-8843	2	2		
KDL-8849	3	3		
KDL-8946			3	2
M-229	~36	1	2	13
M-396	8	2		
MJAV-9009	11	2		6
MNW-8906F	0	0		
MNW-9015			0	

Table 2.4.--continued

Identification number	Dome	Stapes	Tympanic	Mastoid
MSE-9001	12		>5	10
MSE-9104		2	2	2
MSW-032	0		0	
MSW-038	1			
MSW-047	0		0	
MSW-049	1		0	
MSW-050	0		0	
MSW-051	0		0	
MSW-058	0			0
MSW-071	1		0	
MSW-072	1		1	
MSW-081	9		7	
MSW-087				0
MSW-099	1		1	
MSW-102	1		1	
MSW-117			0	
MSW-119	3		4	
MSW-159	0		0	
MSW-166	11	2		7
MSW-169	0		0	
MSW-176	12		>4	
MSW-205	0		0	
MSW-224	2		4	
MSW-227			0	
MSW-228	0	1	0	0
MSW-229	2			2
MSW-235	4		4	4
MSW-236	1		1	
MSW-237	-28		>3	>22
MSW-238	1		0	1

Table 2.4.--continued

Identification number	Dome	Stapes	Tympanic	Mastoid
MSW-239	1		4	
MSW-240	~20			>16
MSW-244	0		0	0
MSW-245	0		0	
MSW-249			0	
MSW-270			1	
MSW-275	7			7
SWFTm-8912B	0	1		
SWFTm-8919	8		2	
UCF-9004			0	0
UCF-9027	1		1	1
UCF-9028	6		6	6
UCF-9049		2	2	2

Table 2.5. Measurements (in percentage) of growth-layer groups of some known-age specimens of Florida manatee in relation to the previous growth-layer group.

Identification number	AC	Last/penultimate (incomplete)	Penultimate/ antepenultimate (complete)	Month of death	Comments
UF 20757	4	10.94	penul > antep	Sep	
SWFTm-9002	3	24.51	91.28	Jan	
UCF-9131	7	39.96	38.74	May	
KDL-8827	7	48.10	67.48	Apr	layers decreasing in width
UF 23993	3	48.38	58.50	Nov	layers decreasing in width
MNE-9102	-19	73.81	49.42	Mar	old, layers closely appressed
SWFTm-7918	11	-----	-----	Dec	last GIG > penultimate
SWFTm-8646	-19	-----	-----	Aug	layers very close together, last > penultimate

Table 2.6. Parameter estimates and test statistics for logistic regression of resorption against age class, length (cm), and sex ($n = 991$)^a.

Variable	Parameter estimate	Standard error	Chi-Square ^b	P
Age class	-15.1546	1.2257	152.8582	0.0001
Length	-7.5817	4.5292	2.8021	0.0941
Sex	0.9873	0.2529	15.2374	0.0001

^a excludes all cases of heavy resorption

^b df = 1

Table 2.7. Examples of minimum ages of living individual Florida manatees recorded in field studies and in captivity. For field studies, CR = Crystal River and BS = Blue Spring. Year of most recent sighting record in field studies is August 1992, except as noted.

CAPTIVES

Individual	Year of capture/ birth	Relative age at first sighting	Current status	Elapsed time
Snooty ♂	1948	born 21 July	living	44 years
Romeo ♂	1957	young adult	living	35 years
Juliet ♀	1958	adult	living	33 years
Rosie ♀	1968	adult	living	24 years

FIELD STUDIES

Individual	Year of capture/ birth	Relative age at capture	Current status	Elapsed time
BS-19 ♂	1970	adult	living	22 years
BS-11 ♂	1970	adult	living	23 years
BS-02 ♂	1970	adult	living	23 years
CR-28 ♀	1967	adult	¹ living	23 years
CR-60 ♀	1967	unknown	² living	24 years
CR-25 ♂	1967	unknown	³ living	24 years
CR-06 ♂	1967	juvenile	³ living	22 years
CR-21 ♂	1967	unknown	¹ living	23 years

¹ through 1990

² through 1991

³ through 1989

Figure 2.1. A. Photomicrograph of whole section (original magnification 10x) and detail (original magnification 100x) from the central portion of the periotic dome of an age-class-8 Florida manatee.

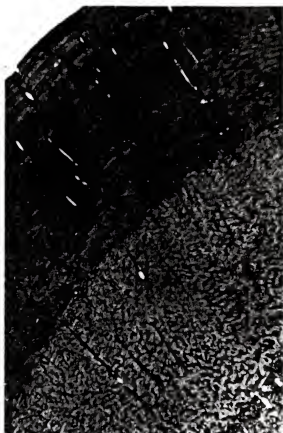


Figure 2.1. B. Line drawing of microscopic structure of the periotic dome in 2.1A. PV, primary vascular bone; SV, secondary vascular bone; CL, compact lamellar bone; WR, white rim. Numerals 1-8 mark the growth-layer groups.

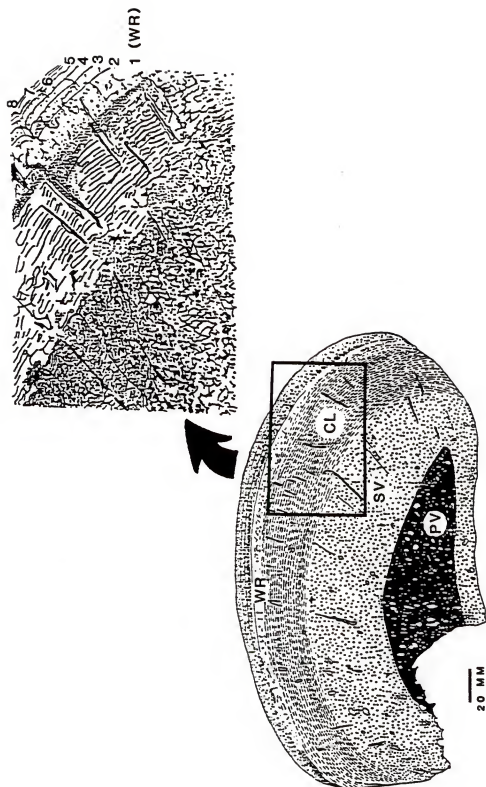


Figure 2.2. Bones examined for growth-layer groups. Dashed lines indicate planes of sections. A, mandible; B, rib; C, pelvic bone; D, humerus; E, radius; F, ulna; G, phalanx; H, carpal bone; I, hyoid bone. Not drawn to scale.

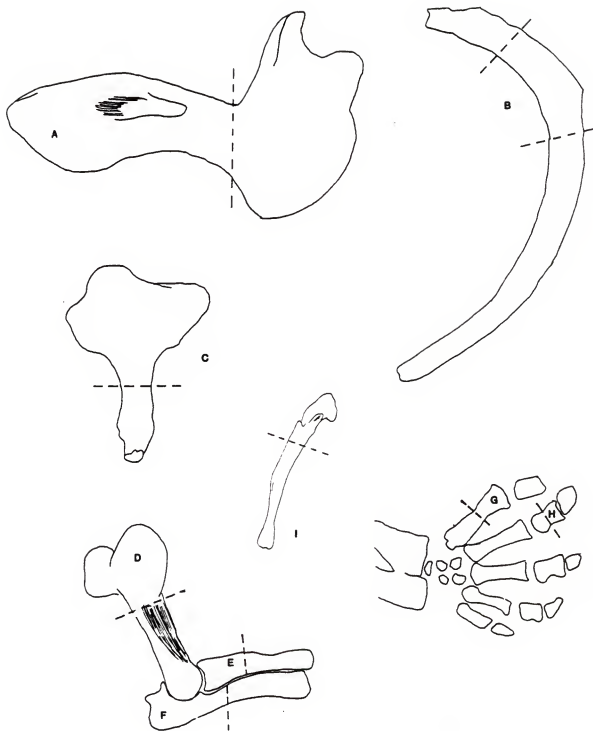


Figure 2.3. Manatee tympano-periotic complex (right side).
A, ventral view; B, stapes. 1 = tympanic bone; 2 =
periotic dome; 3 = mastoid portion of the periotic
bone; 4 = petrous portion of the periotic bone. Dashed
lines represent planes of section.

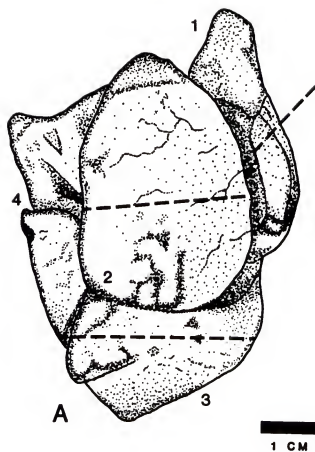




Figure 2.4. Microscopic appearance of bone structure in the periotic dome of a near-term manatee fetus (MNW-8906F). PV, primary vascular bone; SV, secondary vascular bone. Original magnification 40x.

Figure 2.5. A. Thin sections of the peritotic dome of M-431, a 282-cm female of unknown age. GLG, growth-layer group. Original magnification 40x. B. Detail of previous section. Note the pattern of deposition of growth-layer groups with bifurcated layers (BL) and multiple layers (ML). Original magnification 100x.

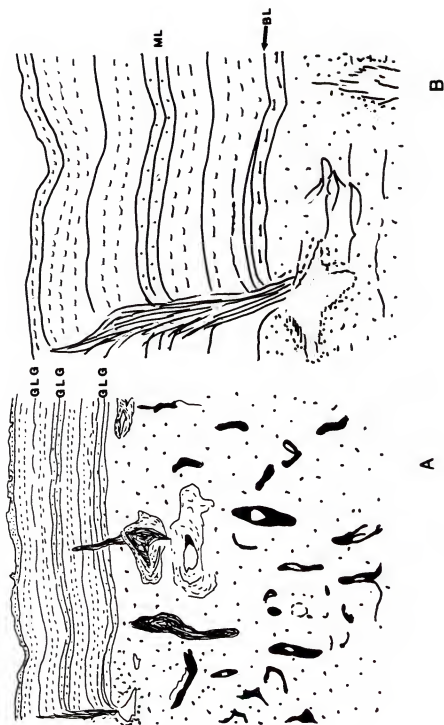
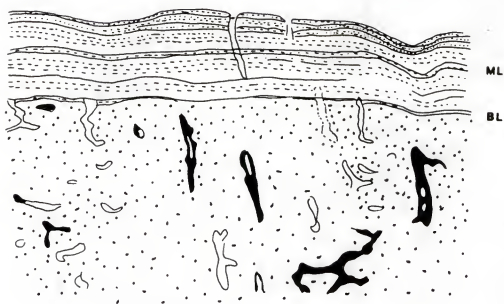
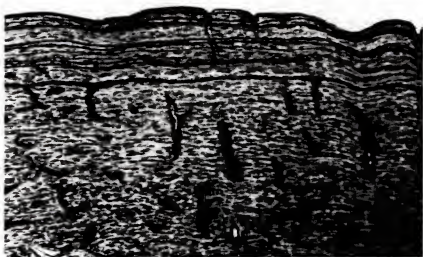


Figure 2.5. C. Photomicrograph and corresponding line drawing of thin section of the periotic dome from UF 15120, a 305-cm male of unknown age. Codes as in Figs. 2.5A and 2.5B. Original magnification 40x.



C



Figure 2.6. Thin section of the periotic dome of a manatee found dead at birth (MSE-8915). Only primary vascular (PV) and secondary vascular (SV) bone are present. Original magnification 10x.

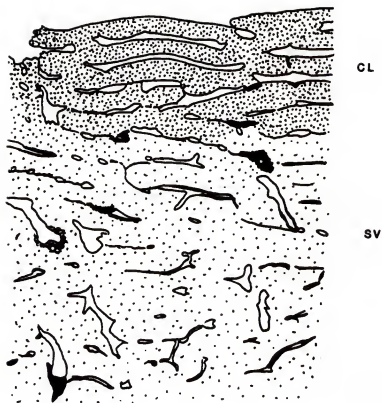


Figure 2.7. Thin section of the periotic dome of a young manatee calf (M-420). Note the transition from secondary vascular (SV) to compact lamellar (CL) bone. Original magnification 20x.

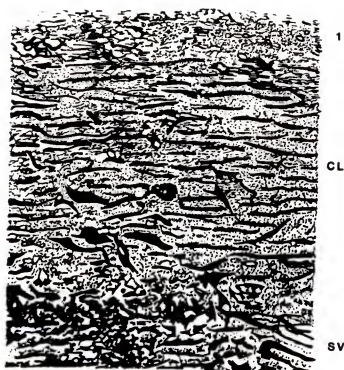


Figure 2.8. Thin section of the periotic dome of an approximately 1-year-old, individually recognizable manatee from Blue Spring (MJAV-8921). Note the transition from secondary vascular (SV) to compact lamellar (CL) bone and the change from regular to irregularly packed vascular canals (first-year zone, 1) at the top of the figure. Original magnification 40x.



Figure 2.9. Thin section of the periotic dome of a 3-4-year-old, individually recognizable manatee from Blue Spring (SWFTM-9002B). First-year zone is represented by numeral 1. Numerals 2 and 3 indicate additional growth-layer groups. Original magnification 20x.

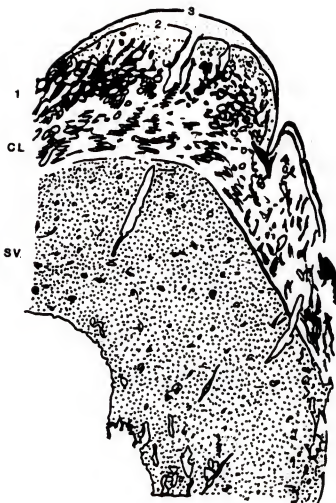


Figure 2.10. Thin section of the tympanic bone of a 2-3-year-old captive manatee (UF 20774). Note the transition from secondary vascular (SV) to compact lamellar (CL) bone, and the first-year zone (1). Numerals 2-3 mark the growth-layer groups. Original magnification 10x.

Figure 2.11. A comparison of ranges of total body lengths of wild, known-age individual manatees from Blue Spring (top) measured alive (O'Shea and Reep, 1990), with total body length and age class of a separate group of dead manatees (bottom). Age classes of dead manatees were estimated based on growth-layer groups in the periotic dome. Solid lines and squares represent ranges and means (respectively). Numbers next to mean value represent sample sizes.

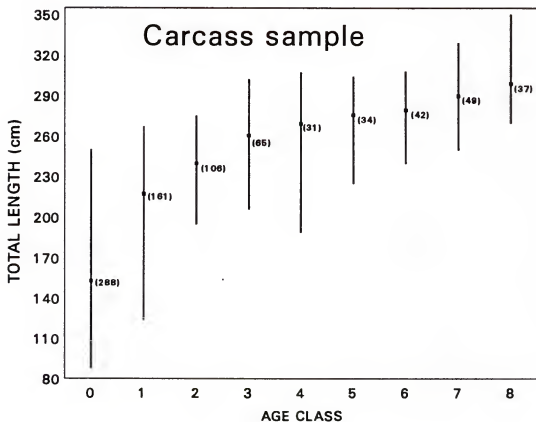
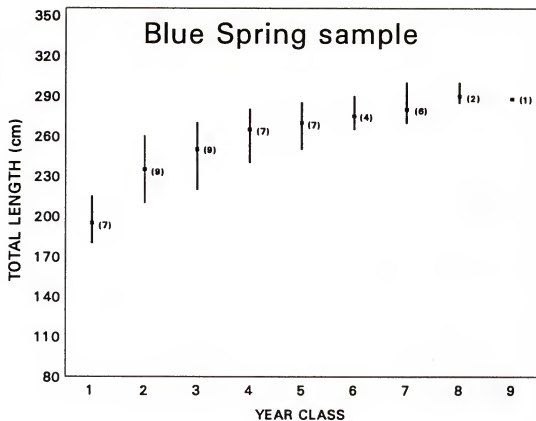


Figure 2.12. Photomicrograph and line drawing of thin section of the periotic dome of a 7.9-year-old, individually recognizable manatee from Blue Spring (KDL-8827). Note the change relative to the first-year zone (1). Numerals 2-7 mark growth-layer groups. Original magnification 20x.

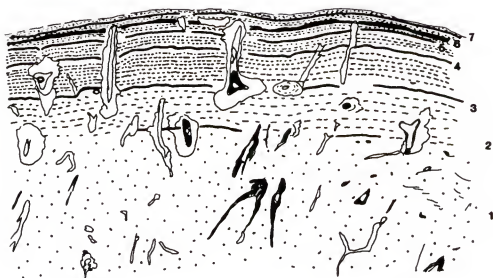
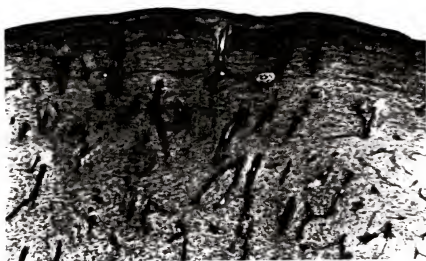
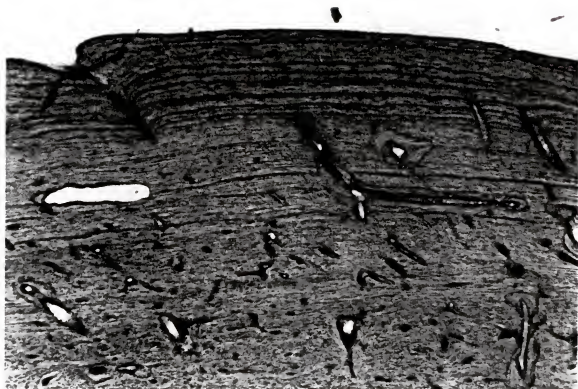




Figure 2.13. Thin section of the periotic dome of an approximately 3-4-year-old, individually recognizable manatee from Blue Spring (UF 23993). Note the change relative to the first-year zone (1). Numerals 2-3 mark growth-layer groups. Original magnification 20x.

Figure 2.14. Photomicrograph of the earbone of a female manatee rescued as a small calf and held in captivity for 9 years (SWFTm-8312B). Original magnification 40x.



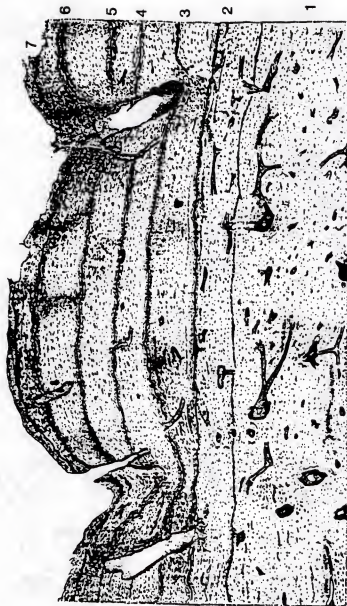


Figure 2.15. Line drawing of a thin section (original magnification 40x) of the periosteal dome of a 7-8-year-old male manatee, recovered as a young calf in 1984 and held in captivity through 1991 (UCF-9131). Numerals mark growth-layer groups.

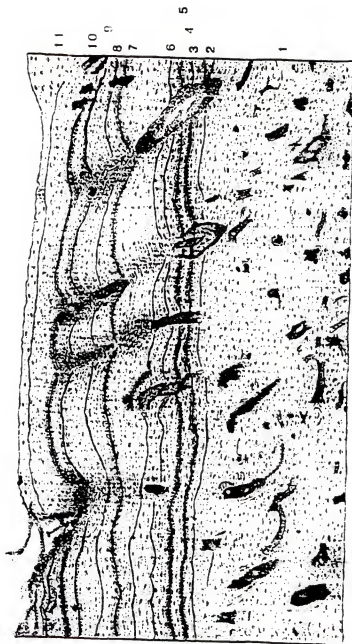


Figure 2.16. Line drawing of a thin section (original magnification 40x) of the periotic dome of a female manatee rescued as a young calf and maintained in captivity for 12 years (SWFTm-7918B). Numerals mark growth-layer groups.

Figure 2.17. Thin section of the periotic dome of an individually recognizable female manatee from Blue Spring (SWFTm-9215B) that had been known as an adult for a minimum of 22 years. Original magnification 40x.

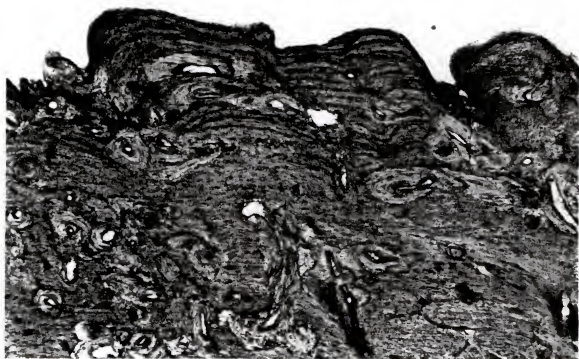


Figure 2.18. Line drawing and photomicrograph of thin section of the periotic dome of a 343-cm female from Blue Spring (MNE-9102), known since 1974. Original magnification 40x.

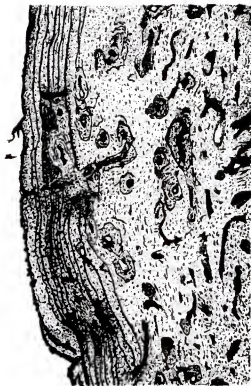


Figure 2.19. Photomicrograph and line drawing of thin section of the periotic dome of an adult, individually recognizable manatee from Blue Spring (UF 20773) known from 1970 to 1982. Note the Haversian systems (HS) and a large erosion bay (EB) indicating resorption. Numerals 2-26 mark growth-layer groups. Adhesion lines corresponding to growth-layer groups 23-26 appear throughout the section as thinner lines than the previous. Original magnification 100x.

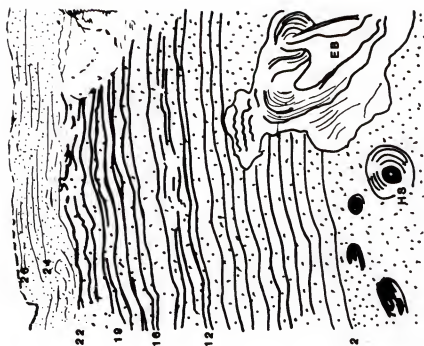




Figure 2.20. Line drawing of thin section of the periosteal dome of a 2-year-old captive-born manatee (UF 20758). Note the change from secondary vascular (SV) to compact lamellar (CL) bone, the change relative to the first-year zone (1), and the increased density towards the periosteal edge. Original magnification 20x.

Figure 2.21. Photomicrograph of the thick section of the periostic dome of UF 20758 under ultraviolet light. The fluorescent band represents tetracycline deposition in the bone tissue. Original magnification 40x.



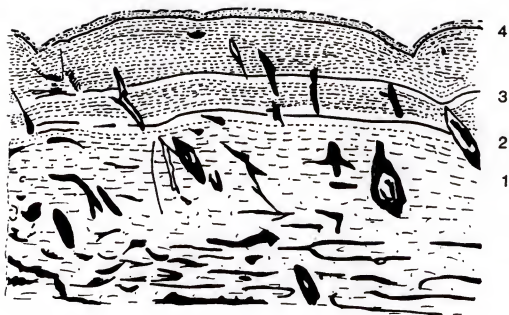


Figure 2.22. Thin section of the periotic dome of UF 20757, a manatee of unknown age, estimated at age class 4. Note the change from regularly to irregularly spaced canals in the first-year zone (1). Numerals 2-3 mark growth-layer groups. Original magnification 40x.

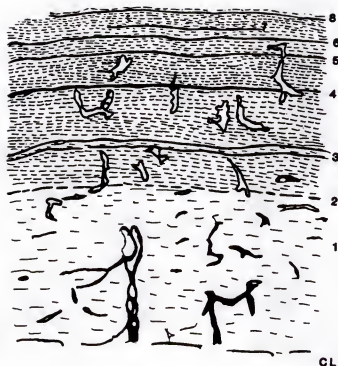


Figure 2.23. Thin section of the periostic dome of M-453, a known minimum-age manatee at least 7.5 years of age. Numerals 2-8 mark growth-layer groups. Growth-layer group 3 is bifurcated. Original magnification 40x.

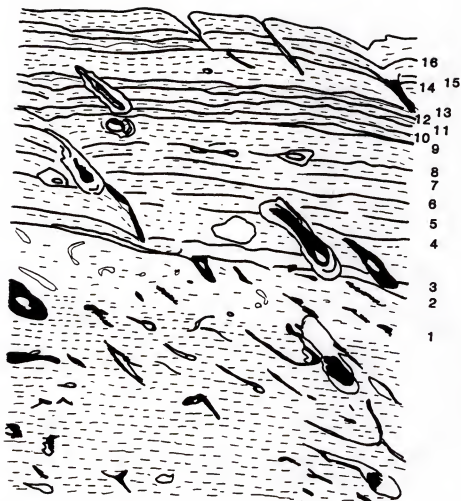


Figure 2.24. Thin section of the periotic dome of UF 24971, a known minimum-age manatee, where known age is at least 9, and age-class estimation is at least 16. Note the change in structure in the first-year zone (1). Numerals 2-16 mark growth-layer groups. Original magnification 40x.

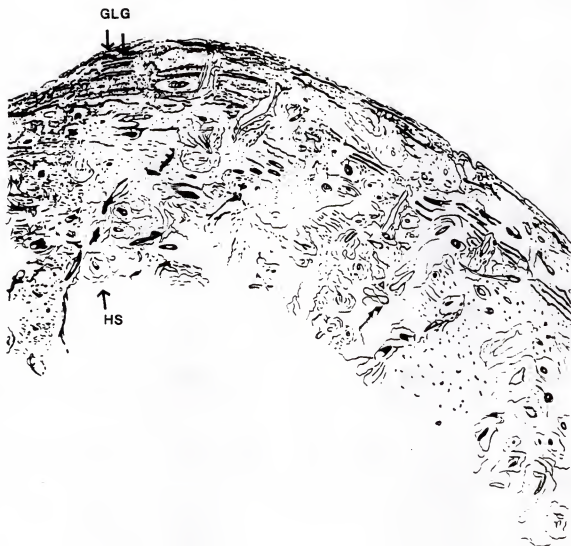


Figure 2.25. A. Thin section of the periostic dome of an adult, individually recognizable manatee from Blue Spring, known to be at least 20 years old (SWFTm-8646B). Note the extensive amount of tissue that has been remodeled by Haversian systems (HS), allowing only a minimum growth-layer-group (GLG) count. Original magnification 20x.

Figure 2.25. B. Photomicrograph and line drawing of detail of Fig. 2.25A, showing the maximum number of growth-layer groups counted. First-year zone not depicted. Original magnification 100x.

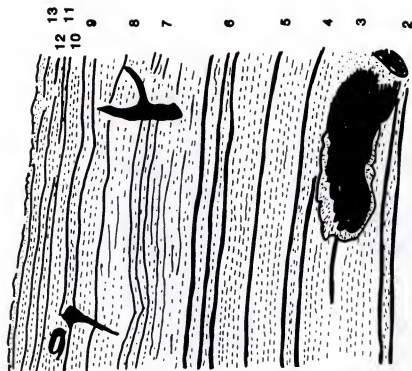




Figure 2.26. Line drawing of the thin section (original magnification 40x) of the periotic dome of a Steller's sea cow *Hydrodamalis gigas* showing a minimum number of layers. Dry preservation and chemical harshness contributed to periosteal damage.

Figure 2.27. Variability in the deposition of band and line pattern in the most recently-formed growth-layer group in Florida manatees. Upper graph includes all animals for which age was estimated. Lower graph represents small calves (≤ 220 cm) classified as age-class-1 based on bone structure. Black columns represent line deposition, and grey columns represent layer deposition.

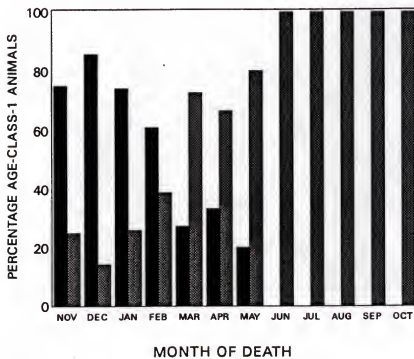
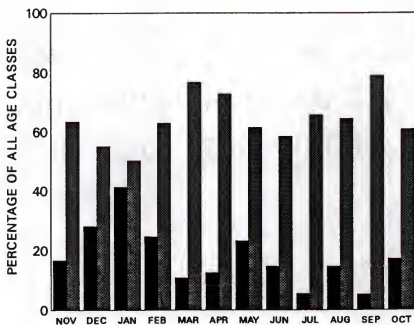


Figure 2.28. Variability among 5 replicates in growth-layer-group counts of 1,078 Florida manatees. Squares indicate the mean variance in readings for each age class. Vertical lines represents maxima and minima of variance and numbers above the lines are sample sizes.

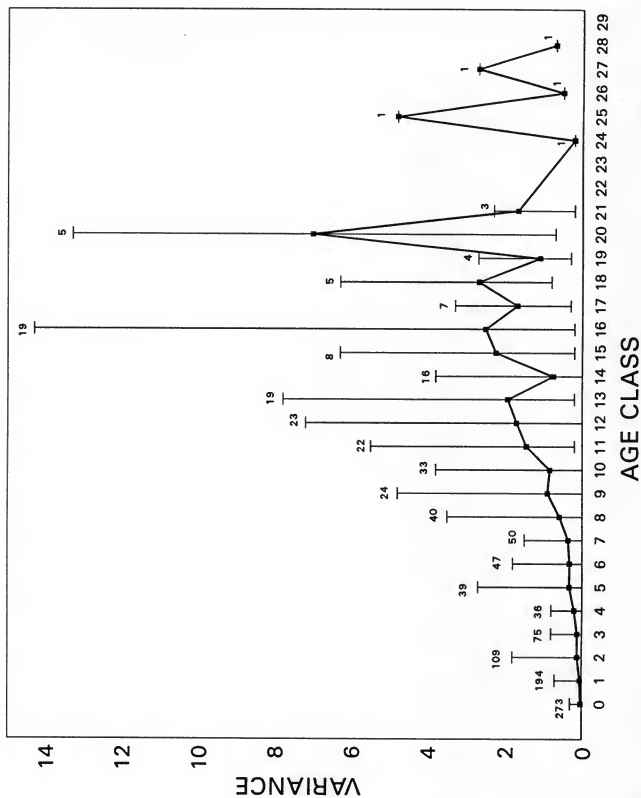


Figure 2.29. Proportions of manatees with no, light, moderate, or heavy resorption of bone in the periotic dome, classified by sex and size category.

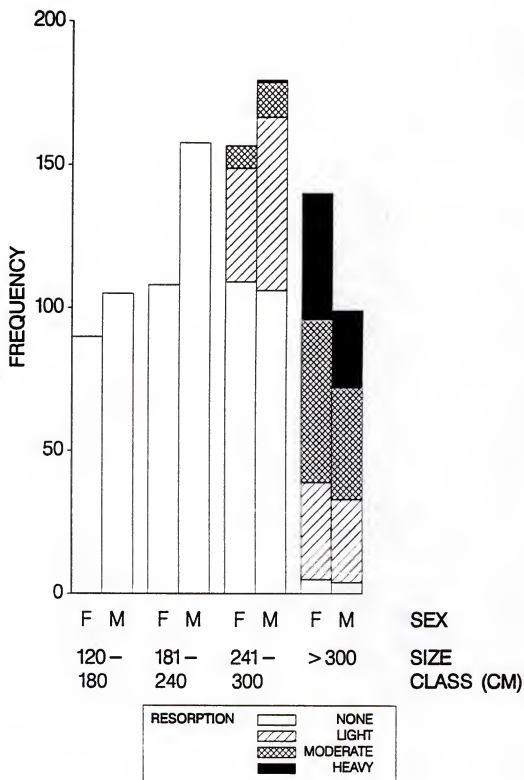
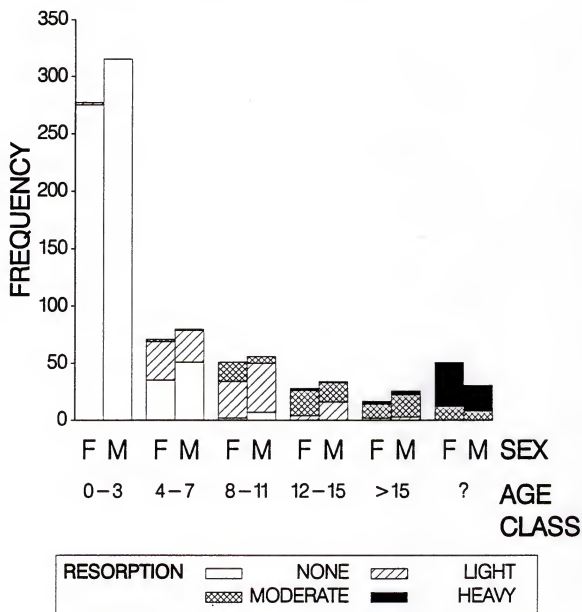


Figure 2.30. Proportions of manatees with no, light, moderate, or heavy resorption of bone in the periotic dome, classified by sex and age category.

Figure 2.31. Photomicrograph and line drawing of thin section of the periotic dome of a 362-cm female of unknown age (UF 15115), estimated at age class ~59. A minimum growth-layer-group (GLG) count was obtained due to the heavy resorption present in the bone. Original magnification 20x.



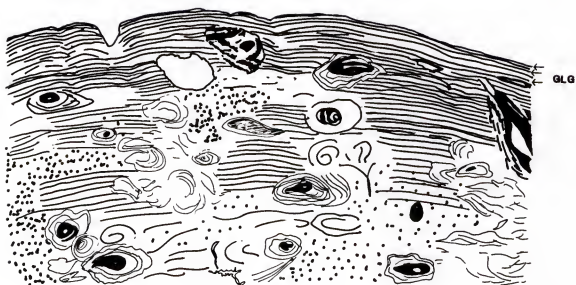
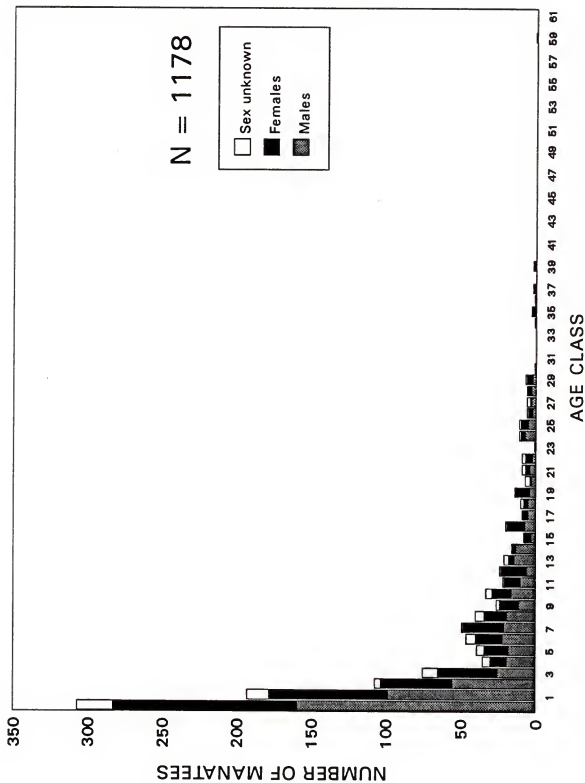


Figure 2.32. Age distribution of Florida manatees recovered dead in the southeastern United States.



CHAPTER THREE

REPRODUCTIVE PARAMETER ESTIMATES
IN FEMALE FLORIDA MANATEES

Introduction

Age at sexual maturation, age at first parturition, and age-specific fecundity represent valuable biological parameters for population modeling and, coupled with mortality, influence the ability of a population to replace itself. Long-term carcass-salvage based studies of manatees in Florida have produced extensive data on biological parameters as well as samples for analyses. The objectives of my study were to match biological data collected over the years on this large sample of manatees with newly derived age-class estimates and describe age-related aspects of reproduction in the female segment of the sample.

Materials and Methods

Specimens used in this study originally comprised all the female manatee carcasses recovered in the southeastern United States between April 1974 and December 1991, by cooperating members of the manatee carcass salvage program. The specimens were measured and processed according to standard procedures (Bonde et al., 1983). During routinely

performed necropsies, various institutions have collected tissue specimens for eventual use in studies of aging and reproduction. Whenever the state of decomposition permitted, a cause of death was designated for each carcass.

Data Censoring

Data collected prior to 1976 were not included in the analyses below because the salvage program was not fully operational in terms of area covered until the end of 1975. Because one of my objectives was to estimate fecundity (defined as live calves born to a female), I reanalyzed data sheets for specimens in the "dependent calf" category for indications of premature death, considering carcass size, level of healing of the umbilicus, inability of lung tissue to float, and presence or absence of meconium in the gastrointestinal tract. Twenty-two specimens were judged either by field examiners or during review of records to be aborted fetuses or stillborn calves. I used these specimens in the calculations of seasonality of reproduction and size at birth, but excluded them from the analyses of reproductive parameters. The revised data set included 821 females, 511 of which had ages estimated through growth-layer-group counts (see Table 3.1 for summary of data).

Age Determination

Age was estimated based on counts of growth-layer groups in the dome portion of the tympano-periotic complex (Marmontel et al., 1990). The technique consists of decalcifying a slab of bone and obtaining 40- μ m thick sections, using a freezing microtome, and subsequently staining the sections with hematoxylin. The method was validated by the examination of 16 known-age, minimum known-age, or tetracycline-marked individuals, showing that one growth-layer group corresponds to 1 year (see Chapter 2). The bone was affected by resorption in 32 cases, allowing the assignment of a minimum age only, representing 22% of the adult females for which earbones were available ($n = 143$). Under these circumstances, a "best estimate" approach was taken, by examining the structure of the bone, the extent and degree of resorption, and the distances between visible layers (see Chapter 2). Minimum and maximum possible ages were assigned, and the midpoint was used as the estimate of age. Five readings were taken for each earbone specimen. With one exception, all females estimated to be of age class ≥ 21 were classified by this minimum-age method.

Reproductive Data

Information on reproductive status was obtained from macroscopic and microscopic examination of reproductive

tracts of 77 females ranging from 115 to 375 cm in total length, as well as review of necropsy sheets, containing information gathered at field examination for 460 females. Information on both age and some measure of reproductive condition was available for 275 females (132 immature and 143 mature, Table 3.1). An assessment of the reproductive condition (mature vs. immature) was obtained in the field from the size of the female (although there are intermediate sizes where this method is not reliable), and size, appearance, and vascularization of the uterus and ovaries. The presence of one or more corpora lutea, a fetus, and milk were used as macroscopic indications of mature, pregnant, and lactating animals, respectively. These three conditions were identified or confirmed histologically (Table 3.2), by examining follicular development and recording the presence of corpora lutea or albicantia in the ovaries, indicative of previous ovulation (Mossman and Duke, 1973).

Reproductive schedules

Estimates of reproductive parameters were first calculated based on a "conservative" schedule, containing solely the confirmed cases of pregnancy and lactation. Alternative schedules were then calculated, to provide a range of reproductive values. Females that according to comments on data sheets, had "recently calved", or calved within a few weeks, were included in the "intermediate" schedule of estimates of fecundity and pregnancy rates.

Cases that noted the possibility of a female lactating (based on size of mammae, or proximity of a small calf) were added to the lactating portion of the sample under the intermediate schedule. Rich superficial vascularization and enlargement of the uterus are indicators of the onset of pregnancy in cetaceans (Benirschke and Marsh, 1984; Collet and Robineau, 1988). Females recorded in necropsy sheets as "possibly" having calved were so classified because of enlarged and vascularized reproductive organs, but without mention of a placental scar to confirm such condition. Therefore, those females could have been pregnant with small fetuses. A great deal of the uncertainty as to the classification of these females could be attributed to the condition of the carcasses. In an "optimistic" schedule I also included females in those two last categories as pregnant. No additional lactating females were added under the optimistic schedule, because all were previously included in the intermediate category. The conservative schedule is believed to err by exclusion of some females that were reproductive and were not so classified; it also derives very low estimates for the reproductive parameters. On the other hand, the optimistic schedule may inflate the estimates by assigning females into reproductive categories based on somewhat subjective assessment of their condition. Therefore, the intermediate schedule is believed to be the most realistic.

Calculation of reproductive parameters

Age-specific fecundity was calculated as the number of female births/female/year (Caughley, 1977), using the formula

$$m_x = \frac{B_x}{2f_x}$$

where f_x is the number of mature females sampled, and B_x is the number females classified as pregnant or lactating. Because manatees calve less frequently than once a year, I adjusted the number of lactating females by an arbitrary factor of 0.4, to account for those females that might have been in their first year of lactation. I chose this value for being intermediate between the possibilities of 1/2 and 1/3 of the lactating females having given birth recently. The range of values for individual years was very wide, so I grouped the data in 2-year intervals to produce a smoother curve and more comparable sample sizes.

Pregnancy rate was expressed as the proportion of pregnant females in the sexually mature sample. Annual pregnancy rates (APR) were calculated as the proportion of pregnant females divided by the gestation period (in years) (Perrin and Reilly, 1984). Assumptions involved are: (1) the distribution of reproductive condition in the sample reflects that in the actual population, (2) there are no

biases due to seasonal calving, and (3) no pregnancies go undetected (Perrin et al., 1977). Some or all of the assumptions may have been violated: (1) sick and non-reproductive females may die at a greater rate than healthy animals, (2) calving is known to be seasonally diffuse, and (3) some pregnancies went undetected for reasons such as decomposition, examiner's experience, or early pregnancy stage. Variance of annual pregnancy rates was calculated as

$$APR * \frac{1-APR}{n}$$

(Perrin and Reilly, 1984). Calving intervals (CI) were calculated as the reciprocal of the pregnancy rate, and their variances as

$$APR^{-4} * \sigma^2_{APR}$$

(Perrin and Reilly, 1984). Seasonality in reproduction was analyzed by examining total body length and month of death of unborn, near-term and term fetuses with chi-square goodness-of-fit tests, and general linear models with a contrast statement.

The gross annual reproductive rate (GARR) was calculated as [(% females in the population)*(% females mature)*(annual pregnancy rate)] (Perrin and Reilly, 1984; Smith, 1983). As an alternative estimate of the gross

reproductive rate, I equated the number of lactating females to the number of calves.

Results

Age Composition of Female Carcasses

The frequency distribution of ages for the sample of females was highly skewed (Fig. 3.1). Considering only those specimens with information on reproductive status ($n = 143$), average age of sexually mature females was 12.6. This was similar to a value of 12.9 years obtained by assuming that all females of age class ≥ 5 are mature, and adding the individual age-class-3 and age-class-4 specimens known to be mature ($n = 208 + 7 + 2 = 217$).

Attainment of Sexual Maturity

Age at sexual maturation

Some females attained reproductive maturity at age class 3, but sample sizes were not large enough to calculate mean age of sexual maturity, defined as the age where 50% of the females are mature (DeMaster, 1978). All female carcasses for which age class was ≥ 5 , and reproductive information was available, were sexually mature ($n = 143$).

I observed seven reproductive females at age class 3 (Table 3.3). A dilated cervix, enlarged uterine horns, and quiescent ovaries observed at necropsy suggested that one of the age-class-3 females (KDL-8852, 283 cm total length) had

recently aborted a calf. Another female (SWFTm-9008B, 302 cm total length, age class 3) died in captivity as a result of pregnancy complications and retention of a dead, full-term fetus. Specimen M-195 (276 cm total length, age class 3) exhibited one placental scar in each uterine horn; judging from age and body size, probably at least one of the pregnancies marked by the scars did not reach term. One placental scar in each uterine horn also was found in M-199 (294 cm, age class 4), but it is not possible to tell if the female carried both pregnancies to term. Specimen UCF-9021 (283 cm total length) was the youngest female carrying a fetus at the time of death, at age class 4, with cause of death independent of reproductive status.

Mature females age class 3 were collected as early as 1978. Six out of the seven reproductive females at age class 3 were recovered on the east coast, between 1978 and 1988; the seventh was found on the west coast in 1990.

Length at sexual maturation

The smallest female classified as mature based on field examination measured 254 cm and was estimated at age class 3. The average length of the seven youngest mature females (age class 3) was 279.7 cm (SD = 14.2; range = 254-302). A 257-cm female (KDL-8811, age class not available) was lactating. The average length of the five immature females at age class 3 was 254.8 cm (SD = 13.6 cm; range = 242-272 cm).

Reproductive Traits of Mature Females

Approximately 46% (212/460) of the specimens whose reproductive tracts were examined in the field or in the laboratory were classified as mature. Forty percent (85/212) of those individuals were either pregnant or lactating at the time of death.

Resting females

Not all mature females were reproductively active. Nine (19%, age classes from 7 to 23) of the 47 mature females examined histologically were neither pregnant nor lactating and did not present signs of ovarian activity (i.e., no large Graafian follicles, corpora lutea or corpora albicantia). Another 18 (38%) were not pregnant, or lactating but had active ovaries (follicular or luteal). Again, based on the histological sample, 43% were reproductive, very close to the overall sample of 40% (85/212). In the larger sample, under the intermediate schedule ($n = 261$, Fig. 3.2), a similarly high percentage (48.6%) of females was in the resting category (non-pregnant, non-lactating). Ovarian activity was not necessarily assessed in all cases: the "resting" category probably included some pregnant or lactating females whose reproductive status was masked by decomposition. In addition, some small embryos may have been missed at necropsy.

Pregnant and lactating females

Placental scars as indication of pregnancies. The maximum number of placental scars ($n = 4$) was found in a 307-cm female (M-82-28, age estimate not available). Only one placental scar was present in each uterine horn of M-85-23, a 355-cm female (age estimate not available). Specimen UF 24989, a known animal from Crystal River (CR09F, "Dihedral", M-422) was estimated at age class 28. She gave birth to a calf in 1979 (Rathbun et al., 1990) and measured 375 cm when she died in 1985. The absence of placental scars in her uterus indicated that scars may be resorbed and disappear sometime after 6 years.

Pregnant-and-lactating females. Simultaneously pregnant and lactating females have been recorded in only two instances (0.9% of the mature sample). The two cases were specimen M-021, recovered dead in April 1976 (299.7 cm, age estimate not available), with a 152-cm fetus protruding from its genital aperture, and M-155 (355 cm, age class ~25), which was carrying a 123-cm fetus when it was found dead in June 1979.

Incidence and seasonality of pregnancy and lactation. The overall percentages of pregnant and lactating females in the mature sample under the conservative schedule ($n = 212$) were 22% and 17.9% respectively (Table 3.4), corresponding to 18.4% (39.9 reproducing x 46.1 mature) of females reproductively active in the overall sample ($n = 460$). It

is probable that these percentages are underestimated: several females were classified during necropsies as "possibly pregnant", "possibly lactating", having "recently calved/aborted" and having "possibly recently calved", or had enlarged reproductive organs. Presence of milk was likewise not recorded, except in two cases where it was noted as absent. According to the intermediate schedule (Table 3.5), the proportion of pregnant females was 33.3%, whereas lactating females account for 18% (Fig. 3.2, $n = 261$). This results in an estimate of the reproductively-active portion of the population of 23.6% (46.1×51.3).

Season of births. The probability of detecting a fetus during gross necropsy increases with the proximity to term simply because of fetal size. Most of the pregnancies (72%) were detected in carcasses either during winter or spring (Fig. 3.3). Data on fetuses detected in carcasses of pregnant females ($n = 43$), aborted fetuses ($n = 14$), stillborn calves ($n = 29$), and calves born alive that died soon after birth ($n = 40$), indicate that the largest of these occur from March to July (Fig. 3.4). Relatively few large, near-term fetuses were observed in the autumn and winter ($\chi^2 = 17.76$, $df = 1$, $P < 0.001$). There was a significant difference between lengths of unborn and aborted fetuses with season ($F = 9.18$, $df = 3$, $P = 0.0001$). There was no difference in lengths of fetuses among warm months ($F = 0.55$, $df = 1$, $P = 0.4606$) and among cold months ($F = 0.04$,

$df = 1$, $P = 0.8353$); however, there was a significant difference between warm (unweighted mean of 99.4 cm) and cold months (unweighted mean of 56.8 cm) ($F = 18.06$, $df = 1$, $P = 0.0001$).

Incidence of twinning. There are two confirmed instances of females simultaneously carrying two fetuses at necropsies (M-331, 375 cm and AC -29, and M-389, 313 cm and AC 7), corresponding to 4% of all pregnancies detected in the carcass sample. Circumstantial evidence suggests three other cases of twinning. Manatees M-190 and M-191 (two female perinatal calves measuring 103 and 110 cm, respectively), MSE-8915 (132-cm female) and MSE-8914 (~130-cm male), and MSW-187 (83-cm female) and KDL-8906 (85-cm male) represent three pairs of dead calves or near-term fetuses found at the same time and at the same location.

Size at birth. The largest unborn fetus was recorded in April 1976 and measured 152 cm, or 50.7% of its mother's body length (M-021). The largest newborn (still with its umbilical chord attached) was 160.0 cm in length. Based on lengths of stillborn calves and calves born alive but dying soon afterwards ($n = 69$, Table 3.7), length at birth ranged from 80 to 160 cm, with a mean of 122 cm ($SD = 17.1$ cm).

Reproductive Parameters

Age-specific fecundity

Age-specific fecundity increased from zero (age class 0) to 0.27 at age of first parturition (age classes 4-5),

and remained at a level of approximately 0.24 (Fig. 3.5) under the intermediate schedule (age classes 4 through 29; Table 3.8). This schedule contained values for age class 3 (4 females with indication of pregnancy or parturition, but see Age at sexual maturation), which were not included in the calculation of mean age-specific fecundity. Age-specific fecundity values did not differ across the age classes for either schedule (Table 3.9). Under the conservative and optimistic schedules, mean age-specific fecundity values were calculated as 0.19 and 0.27, respectively (Table 3.8).

Length of reproductive life and senescence

There was no identifiable peak of reproductive potential or decline in reproductive activity with age, although sample sizes were small in the older age classes. Close gross examination of 11, and histological processing of 37 reproductive tracts from mature female manatees failed to indicate signs of senescence. UF 24989 (M-422, CR09F), an extremely large (375 cm, 1,650 kg) female with an estimated age class of 28, had four fluid-filled cysts, few and very regressed corpora albicantia in its ovaries, and the ovarian surface appeared generally smooth, as if exhausted of follicles. Nevertheless, there were 16 and 8 corpora lutea in the right and left ovaries, respectively, indicating that she was still ovulating. Other observations also suggest that manatees remain reproductive into old age.

"Juliet", brought to Miami Seaquarium as an unknown-aged adult in 1958, is still reproductively active after 34 years in captivity (Odell et al., 1992). Specimen M-331 (375 cm) estimated at age class ~29 was carrying twins when she died as a result of a collision with a boat in April 1983. Female KDL-8867, 332 cm at death and estimated at age class ~35, had calved a few weeks prior and was lactating. Manatee KDL-8745 (age class ~39) was "well nourished", had recently calved and was lactating when she drowned in a shrimper's net. Based only on these two old reproductive females (KDL-8867 and KDL-8745), minimum estimates for the coefficient of female fertility (B, maximum length of breeding period) (Braham, 1984) are 0.55-0.60 life span, given an assumed maximum life span of 60 years (Chapter 2).

Annual pregnancy rates

Over half of the mature females (1976-91) were reproducing (including simultaneously pregnant and lactating) under the intermediate schedule (Table 3.5). The proportion of pregnant females (Fig. 3.3) varied from 16.7% (1976-77) to 41.9% (1984-85). A trend between the probability of female pregnancy and biannual period could not be detected by logistic regression under the conservative schedule ($P = 0.1$ for 8 biannual groups and 16 individual years), but was detected ($P = 0.0001$) under both intermediate and optimistic schedules for 8 biannual periods. If the period 1976-77 is excluded from the

analysis (due to uncertainty as to the representativeness of the sample) a trend is still present for 7 biannual groups ($P = 0.0388$) and for 14 individual years ($P = 0.005$).

Annual pregnancy rates spanned a two-fold increase (from 0.190 to 0.394), depending on the schedule (conservative, intermediate, or optimistic), and gestation period considered (Tables 3.10-3.12). The range of gestation estimates was selected based on field and captive studies (Odell et al., 1992; Rathbun et al., in manuscript), with 12 months being the most likely interval (Dekker, 1977; Rathbun et al., 1992). Under the intermediate schedule, average annual pregnancy rate was 33.3% (Table 3.11). The percentage of reproductive females was lowest during the interval 1976-77. This low value was probably due to a time-sampling effect; 1977 began with a very severe winter, and an unusual number of carcasses was recorded (Irvine et al., 1981). Some of these were not thoroughly examined and a large number were badly decomposed. In addition, expertise in recognizing reproductive conditions may not have been fully developed by the various salvage workers early in the program. Other environmental conditions may have influenced the overall temporal fluctuation in pregnancy rates, but are difficult to evaluate with available data.

Calving intervals

The overall calving interval value for eight 2-year periods under the intermediate schedule and assuming a 12-month gestation period was 3.0 years (Table 3.11). With a calving interval of 3.0 years, and total number of 36 reproductive years (for $B = 0.6$), a female manatee would on average produce 12 calves during her lifetime. Assuming a calving interval of 2.5 years (Rathbun et al., in manuscript), the potential maximum number of calves produced by a female manatee would be 14.

Recruitment rate

The gross annual reproductive rate (GARR) represents the annual rate of calf production, or a measure of recruitment into the total population (rather than into a specific age class) and assumes that no mortality takes place over the time of the estimate (Braham, 1984; Perrin and Reilly, 1984).

The average recruitment rate ranged from 0.044 to 0.090 under the 3 schedules, with a mean value of 0.077 under the intermediate schedule and 12 months gestation (Table 3.11).

Estimating GARR as the equivalent of lactating females (percentage of calves) on one hand excludes the calves that did not survive the neonatal period, but on the other hand includes larger, still dependent calves, potentially overestimating the true value of the parameter. The proportion of lactating females in the sample was 18%.

Antillean Manatee

In addition to the larger sample, 13 earbones from female manatees recovered in Puerto Rico were made available through the Caribbean Stranding Network. These included animals from 142 to 300 cm total length. A large female (NEP-ST-164), estimated at age class 25 when it died in May 1991, had recently been pregnant.

Discussion and Conclusions

Attainment of Sexual Maturity

Three criteria are usually considered in determining age at sexual maturation: (1) first ovulation, (2) first conception, and (3) first lactation (Perrin and Donovan, 1984).

Ovulation rate

Ovulation rate may be used as a parameter when modeling reproduction as long as each ovulation results in one corpus luteum and all corpora albicantia remain throughout life, leaving a permanent record of the number of times a female has ovulated (Perrin and Donovan, 1984). One would also have to know if all ovulations resulted in pregnancies, and if not, what proportion was successful. Similar to dugongs (Marsh et al., 1984a), manatees are polyovular, with an average of 36.3 corpora lutea per ovary per pregnancy. If the corpus luteum produces only small amounts of

progesterone, multiple corpora lutea may be necessary to reach progesterone levels high enough to support gestation (Marmontel, 1988). Furthermore, in manatees corpora albicantia do not remain visible for life and are resorbed at an unknown rate (Marmontel, 1988). Females may become pregnant when they first ovulate, but not every ovulation will result in fertilization, so actual pregnancy rate is probably lower than ovulation rate. In addition, the number of corpora varies among individuals, and the ovulation rate may change with age. Therefore, the "first-time-ovulators" method (DeMaster, 1984) cannot be used as an index of sexual maturity, and the counts of corpora albicantia do not reflect the number of ovulations.

Infertile estrous periods

The presence of corpora lutea in nulliparous females indicates that female manatees may undergo a number of ovulations before they first conceive (Marmontel, 1988). This may partially explain the high percentage of free-ranging females that were observed in mating herds but were not observed with an accompanying calf the following season (Rathbun et al., in manuscript). Based on observations of estrous herds containing cows and calves at Crystal River, Hartman (1979) was the first to suggest the occurrence of infertile estrous periods in manatees. Unsuccessful cycles of reproductive activity occur among other mammals before females become active breeding members of the population,

pending full development of the anatomical structures (Bryden and Harrison, 1986; Buechner et al., 1966; Martin, 1981; Newson, 1966; Porton et al., 1987; Sikes, 1971; Sowls, 1966).

Counting placental scars

Another technique used to assess the number of pregnancy events in mammals is to count placental scars (Laws, 1967; Lindström, 1981; Martin et al., 1976; McEwen and Scott 1957; Seber, 1973). Whereas in the related dugong the scars persist for at least several years and their number increases with age (Marsh et al., 1984a), in manatees these structures appear neither to remain visible for long nor to persist throughout life (Marmontel, 1988). The number of placental scars in manatees does not increase with total length or age of the animal. Scars disappear with time and, therefore, cannot be used to estimate the number of parous events in Florida manatees.

Age at sexual maturation

Results from my study indicate that female manatees in the wild sometimes begin to breed between 3 and 4 years of age. The finding of an age-class 4 pregnant female suggests that the animal conceived while at age class 3. This agrees quite closely with a value of 3.2 years calculated for the mean age at sexual maturity of wild animals at Crystal River (Rathbun et al., in manuscript).

Life history theory predicts that long-lived animals have a delayed age of sexual maturation. However, some large mammals (bears, lions, whales) mature earlier than would be expected (Stearns, 1992). That may be explained by the fact that animals well fed may grow and mature rapidly. This may help explain why some manatees reach adult size by age class 3.

Some manatees may conceive as early as age class 3. The anatomical observations detailed in the Methods section are corroborated by observational studies of living animals in the field by Rathbun et al. (in manuscript). However, incomplete pregnancies (see Results) indicate that not all females in the younger age classes are able to carry a pregnancy to term, because of either anatomical or physiological constraints, such as accumulating enough fat reserves to supply long nourishment to the offspring. The large proportion of young age-class females in the carcass sample may partially explain the increased percentage of perinatal deaths in recent years (Ackerman et al. 1992). If more young females conceive, but do not successfully deliver offspring, one would expect a higher early-age mortality rate. On the other hand, a higher calf mortality would tend to decrease the calving interval due to a shortening of the lactation anestrus.

A reduction in the age of sexual maturity is believed to represent a density-dependent mechanism for populations

under increasing exploitation to maintain a net reproductive rate greater than zero (Fowler, 1981b; Smith, 1983).

Numbers of manatees recovered dead have steadily increased since 1974 (Ackerman et al., 1992), and earlier studies have suggested sexual maturity being reached at older ages than reported here (Hartman, 1979; Odell et al., 1981; Rathbun and Powell, 1982). However, these earlier studies were based on much smaller sample sizes. In my study, mature females of age class 3 were collected as early as 1978, and the data I present do not support arguments for changes in the age at sexual maturation over the time period analyzed. Likewise, it would be premature to compare ages at attainment of sexual maturity for manatees occupying the two coasts of the Florida peninsula.

The minimum age at first calving should be about 1 year after the age of first maturation, given a gestation period of approximately 12-14 months (Hartman, 1979; Odell et al., 1992; Powell and Waldron, 1981; Rathbun and Powell, 1982; Rathbun et al., in manuscript). The youngest female in my sample carrying a fetus, and whose cause of death was not related to pregnancy or parturition, was estimated to be between 4 and 5 years of age.

Length at sexual maturation

Knowledge of the body length at sexual maturity in marine mammals allows an evaluation of specimens for which the only available age-related datum is length (Perrin and

Reilly, 1984). The average length at sexual maturity calculated in this study (280 cm) is supported by the relationship between body size and the area of germinal epithelium of the manatee ovary (as verified in dugongs by Marsh et al., 1984a). In manatees the area of germinal epithelium increases gradually until animals reach about 280 cm total length, when a sudden increase occurs, probably due to the presence of the corpora of ovulation and/or pregnancy (Marmontel, 1988). The value is also comparable to the figure derived by Odell et al. (1981) for female size at maturity (260 cm) based on ovary weights.

Reproductive Activity and Senescence

Proportion of reproductive females

The calculated percentage of reproductively-active females (23.6%) conforms well with Rathbun and Powell's (1982) estimate based on aerial surveys and carcass analysis. Under the optimistic schedule, the proportion of pregnant females is approximately 20% higher than for the intermediate schedule (Table 3.6).

Both cases of pregnant females that were concurrently lactating involved a large fetus, suggesting that milk was present for nursing an imminent birth, not that the female had become pregnant while nursing an earlier calf. This further implies that female manatees cannot give birth more frequently than at least every two years.

An increase in annual pregnancy rate is expected to occur in populations under exploitation, as occurs in cetaceans (Fowler, 1984; Laws, 1961; Mackintosh, 1942), pinnipeds (Bowen et al., 1981), and coyotes (Knowlton, 1972). The proportion of reproducing females apparently almost doubled in the period 1976-91. If adult mortality was excessive, it might have been compensated by an increase in reproductive rate. However, cases of density-dependence often occur with populations at carrying capacity, and entail a greater availability of food resources after reduction in numbers. There is no evidence that manatees are at carrying capacity in Florida. Furthermore, the density-dependence concept would be based on decreased manatee density. Although population estimates are unavailable, certain indices indicate precisely the opposite, i.e., populations may have been increasing in recent years. Trend analysis on the counts of manatees in power plant aggregations during winter, when adjusted for temperature, indicated a significant increase in counts on the east coast (Garrott et al., 1992). The relationship between counts and population size is unknown but this finding does not support a hypothesis of population decrease. At least 2 other wintering areas have been shown a remarkable increase in numbers since the late 1970s, even though some of that increase may be attributable to immigration from other sites in Florida (O'Shea, 1988).

Therefore, it is possible that the differences in the proportion of reproducing females over the years is also reflective of differences in other investigators' abilities to recognize reproductive status, in which case no density-dependence mechanism would be at work. Another corroborating factor would be if the cow-calf ratios recorded on aerial surveys also increased over the years. Future research should also examine such data.

Incidence of twins. Manatees usually give birth to single calves, but twins may occur (Odell et al., 1992; Rathbun et al., in manuscript). Survival of twins depends on the anatomic capacity of the uterus, the mother's capability to carry the double pregnancy to term, and her ability to adequately nourish and otherwise care for the offspring. Incidence of twins in the carcass sample (4%) was similar to the proportion reported by Laws (1967) for African elephants, but slightly higher than the percentage obtained based on nursing calves observed in the wild in a larger sample (Rathbun et al., in manuscript) and higher than the norm for uniparous mammals (Laws, 1967; Slijper, 1966). The death of one or both twins near birth might explain the discrepancy between field observation samples and carcass samples. Twinning has also been reported in the Antillean subspecies (Charnock-Wilson, 1968; Gumilla, 1963).

Seasonality of reproduction

Although pregnancy occurs year-round, the data indicate that most pregnancies result in calves born in spring and early summer. Irvine and Campbell (1978) reported that lower numbers of calves seen during aerial surveys in winter compared to numbers observed during the summer are a positive indication of a spring calving season. Apparent seasonality in numbers of observations of mating herds and the fact that births were never observed in winter at warm-water refuges during 15 winters of field work at Crystal River (Rathbun et al., in manuscript) further support this conclusion. Furthermore, Hernandez et al. (in manuscript) indicate virtual cessation of spermatogenesis in winter. Seasonal or diffusely seasonal reproduction has also been suggested for Amazonian manatees (Best, 1982) and dugongs (Marsh et al., 1984a). In comparison with winter, mild spring and summer temperatures would reduce the energetic stress on lactating females and newborn calves, especially favoring greater survival of the young. Breeding and calving in spring and summer may constitute an advantage for both cows and calves. On one hand, vitamin A and protein, necessary for ovulation, are found in green vegetation (Hart and Guilbert, 1933; Miller et al., 1942); on the other hand, young manatees would find nutritious forage to feed on as they start nibbling at a few weeks of age (Husar, 1977). A seasonal reproductive pattern associated with rainfall and

consequent availability of vegetation has also been shown in elephants and other equatorial species (Buss, 1990; Smith and Buss, 1973).

Age-Specific Fecundity and Senescence

In most mammals, fecundity rate rises from puberty and levels off into a plateau (Caughley, 1977). Fecundity is apparently constant throughout the life of a female manatee. Nevertheless, Caughley (1977) pointed out that deviations from a constant rate in adult fecundity can only be detected if large sample sizes are available.

Senescence has only been documented in a few species such as man (Asdell, 1964), large primates (Graham, 1986), some cetaceans (Marsh and Kasuya, 1984; Perrin et al., 1976, 1977), and elephants (Laws et al., 1975). In cetaceans, senescent ovaries are described as lacking follicles, corpora lutea, and young or partially regressed corpora albicantia (Perrin and Donovan, 1984). Changes in ovulation rates, also indicative of senescence, cannot be detected in manatees due to the polyovular condition of their ovaries. Fluid-filled ovarian cysts are associated with inactive ovaries in dugongs (Marsh et al., 1984a). However, in manatees they were always associated with active ovaries, pregnancy, or recent birthing. One captive female has continued to reproduce throughout a 34-year history at Miami Seaquarium (Odell et al., 1992). Although maximum longevity

in manatees may be high (see Chapter 2), only 19 (3.7%) of all females analyzed ($n = 511$) were older than 25 years.

Calving Intervals

Ovulation is suppressed physiologically during lactation, but field and captive observations show that as soon as calves are weaned (or lost) the female may cycle again (Hartman, 1979; Odell et al., 1992). Considering a calf dependency period of 1-2 years (Hartman, 1979), and a gestation period of approximately another year, the value of 3.0 is slightly higher than the interval calculated for free-ranging manatees by Rathbun et al. (1992) at Crystal River and Reid et al. (1992) (but with smaller sample sizes) on the Atlantic coast. It is important to point out that northwestern Florida may represent a particularly favorable calving and rearing area, and the rates derived for that region may not necessarily represent the situation throughout the state. Calving rates may be influenced by age and health of females (a potentially large variable, due to non-fatal boat strikes), and factors such as cows not recycling immediately after the calf is weaned, ovulations not leading to conception, or incomplete pregnancies. Calving intervals are, in addition, time-specific and influenced by the variability in pregnancy rates, and subject to the biases associated with sampling. The actual number of offspring is constrained by the rate of mortality

acting upon reproductive and pre-reproductive age classes. It is also constrained by non-lethal injuries or diseases that cause estrous cycling to be disrupted. The calculated average production of 12 calves per lifetime in manatees corresponds well with the norm for mammals (Eisenberg, 1981).

Recruitment Rate

The recruitment value represents an approximately 8% increase in the population (birth rate), not accounting for mortality associated with young calves. The annual reproductive rate is sensitive both to the percentage of mature females and annual pregnancy rate, so some bias towards a low value might be expected due to violations of the assumption that the sample was representative (due to decomposition or observer-missed pregnancies). The percentage of lactating females, with which I equated calves, was 18%. Hence, the real recruitment rate likely lies between 8 and 18%. Only with accurate accounting of reproductive females in the population can the uncertainty associated with this estimate be removed. Field studies and aerial surveys provide a similar range of estimates. Rathbun et al. (1992) produced an average of 13.5% calves in the Crystal River population, which includes both first- and second-year calves. The average percentage of calves in aerial censuses over a 15-year period was 9.6% (Reynolds,

1992), accounting only for calves one-half the length of a closely associated animal. Low reproduction and long life span are not unique to manatees, but are part of the life-history strategies of some birds (Amadon, 1964; Mertz, 1971), many species of whales (Ridgway and Harrison, 1985), several herbivores (Goddard, 1970; Laws et al., 1975; Verheyen, 1954), and a few carnivorous mammals (Nowak, 1991; Ridgway and Harrison, 1981). Some requisites associated with low rates of reproduction include a stable environment, approximately permanent geographic ranges, and freedom from ongoing forms of mortality such as predators. Slowly reproducing species are not good colonizers and could not recover quickly following a crash or a massive destruction (Mertz, 1971).

Caution must be exercised in interpreting the reproductive estimates, because of biases that would result if the carcass sample is not representative of the total female manatee population.

Table 3.1. Reproductive status of female Florida manatees collected between 1976 and 1991 in the southeastern United States.

Reproductive status		Total number	Number aged	Number not aged
Immature		248	132	116
Mature (n = 212)	Pregnant	47	33	14
	Lactating	38	27	11
	Resting	127	83	44
Status unknown		361	236	125
Total		821	511	310

Table 3.2. Summary of histological characteristics of ovaries, uteri and mammary glands of female Florida manatees in different reproductive stages. CA = corpus albicans, CL = corpus luteum, LGF = large Graafian follicle, PS = placental scar.

Stage	Ovaries	Uterus	Mammary gland
Immature, prepubescent	Pinkish, smooth and flattened surface. Numerous small follicles on the surface	Glands undeveloped	Inactive
Immature, maturing	Several large Graafian follicles	Early proliferative endometrium	Inactive
Sexually mature, nulliparous	Follicular: several LGF, may have CL	Proliferative endometrium, PS absent	Inactive
Ovulating	Luteal: LGF, CL	Secretory endometrium, PS present or absent. Not pregnant	Inactive
Parous	Follicular or luteal	Secretory endometrium, PS	Inactive
Lactating	Luteal: no LGF, several CL	Secretory endometrium	Active
Resting	Resting: no LGF, no CL, some CA	Involuting endometrial glands. PS present or absent	Inactive

Table 3.3. Specimens of *Trichechus manatus latirostris* estimated at age class 3 and classified as mature at necropsy or in laboratory.

Identification number	Length (cm)	Year of death	Coast
NM 527926	254.0	1978	E
M-195	276.0	1980	E
UF 20598	277.0	1985	E
NM 530312	281.0	1978	E
KDL-8852	283.0	1988	E
KDL-8849	285.0	1988	E
SWF-9008B	302.0	1990	W

Table 3.4. Conservative schedule: reproductive condition of 211 adult female Florida manatee carcasses collected in the southeastern United States (1976-91) (numbers in parentheses represent proportion of the total). Number pregnant includes 2 females simultaneously pregnant and lactating.

Year	Total	Resting	Pregnant	Lactating
1976-77	34	29 (0.853)	4 (0.118)	1 (0.029)
1978-79	11	9 (0.818)	2 (0.182)	0
1980-81	16	11 (0.687)	4 (0.250)	1 (0.062)
1982-83	26	15 (0.577)	6 (0.231)	5 (0.192)
1984-85	27	13 (0.481)	9 (0.333)	5 (0.185)
1986-87	26	11 (0.423)	6 (0.231)	9 (0.346)
1988-89	40	23 (0.575)	8 (0.200)	9 (0.225)
1990-91	32	16 (0.500)	8 (0.250)	8 (0.250)
Pooled	212	127 (0.599)	47 (0.222)	38 (0.179)

Table 3.5. Intermediate schedule: reproductive condition of 260 adult female Florida manatee carcasses collected in the southeastern United States (1976-91) (numbers in parentheses represent proportion of the total). Number pregnant includes 2 females simultaneously pregnant and lactating.

Year	Total	Resting	Pregnant	Lactating
1976-77	36	29 (0.805)	6 (0.167)	1 (0.028)
1978-79	15	9 (0.600)	3 (0.200)	3 (0.200)
1980-81	21	11 (0.524)	7 (0.333)	3 (0.143)
1982-83	29	15 (0.517)	9 (0.310)	5 (0.172)
1984-85	31	13 (0.419)	13 (0.419)	5 (0.161)
1986-87	35	11 (0.314)	14 (0.400)	10 (0.286)
1988-89	54	23 (0.426)	19 (0.352)	12 (0.222)
1990-91	40	16 (0.400)	16 (0.400)	8 (0.200)
Pooled	261	127 (0.486)	87 (0.333)	47 (0.180)

Table 3.6. Optimistic schedule: reproductive condition of 286 adult female Florida manatee carcasses collected in the southeastern United States (1976-91) (numbers in parentheses represent proportion of the total). Number pregnant includes 2 females simultaneously pregnant and lactating.

Year	Total	Resting	Pregnant	Lactating
1976-77	39	29 (0.743)	9 (0.231)	1 (0.256)
1978-79	15	9 (0.600)	3 (0.200)	3 (0.200)
1980-81	22	11 (0.500)	8 (0.364)	3 (0.136)
1982-83	32	15 (0.469)	12 (0.375)	5 (0.156)
1984-85	36	13 (0.361)	18 (0.500)	5 (0.139)
1986-87	37	11 (0.297)	16 (0.432)	10 (0.270)
1988-89	60	23 (0.383)	25 (0.417)	12 (0.200)
1990-91	46	16 (0.348)	22 (0.478)	8 (0.174)
Pooled	287	127 (0.442)	113 (0.394)	47 (0.164)

Table 3.7. Morphometrics of unborn and newborn Florida manatees collected in the southeastern United States (1976-91).

Stage	Length (cm)			Weight (kg)		
	minimum	maximum	mean	standard deviation	n	mean
Unborn fetus	1.0	152.0	83.5	42.3	43	1.0
Aborted fetus	42.0	112.0	75.3	18.1	42	1.4
Stillborn calf	80.0	152.0	121.0	15.3	29	9.0
Live birth	82.0	160.0	122.7	18.5	40	8.0
						25.8
						9.8
						24

Table 3.8. Age-specific fecundity and standard error (SE) for female manatees in age classes 2-3 through 38-39 for animals collected in the southeastern United States (1976-91): conservative, intermediate, and optimistic schedules.

Age classes	Conservative		Intermediate		Optimistic	
	Mean	SE	Mean	SE	Mean	SE
2-3			0.183	0.032	0.183	0.032
4-5	0.150	0.045	0.267	0.037	0.285	0.035
6-7	0.145	0.016	0.219	0.013	0.228	0.013
8-9	0.174	0.016	0.211	0.015	0.256	0.014
10-11	0.115	0.016	0.180	0.015	0.233	0.014
12-13	0.150	0.025	0.173	0.025	0.212	0.024
14-15	0.314	0.066	0.314	0.066	0.355	0.053
16-17	0.200	0.036	0.225	0.035	0.225	0.035
18-19	0.175	0.047	0.240	0.043	0.264	0.040
20-21	0.175	0.095	0.175	0.095	0.314	0.066
22-23	0.000	0.000	0.125	0.041	0.125	0.041
24-25	0.240	0.085	0.271	0.063	0.271	0.063
26-27	0.500	0.500	0.500	0.500	0.500	0.500
28-29	0.125	0.083	0.200	0.080	0.250	0.072
30-31						
32-33						
34-35	0.100	0.150	0.233	0.141	0.233	0.141
36-37						
38-39	0.200	0.160	0.350	0.238	0.350	0.238
Average AC 4-29	0.189		0.238		0.271	

Table 3.9. Chi-square results: age-specific fecundity under conservative, intermediate and optimistic schedules. Mean was calculated from age classes 4 through 29.

Schedule	Mean	χ^2	df	P
Conservative	0.189	11.994	12	0.446
Intermediate	0.238	6.759	12	0.873
Optimistic	0.271	7.297	12	0.837

Table 3.10. Conservative schedule: reproductive parameters, based on a gestation period of 12 months ($T_0=1.0$), 13 months ($T_0 = 1.08$) and 14 months ($T_0 = 1.17$) for female manatees collected in the southeastern United States (1976-91). APR = annual pregnancy rate, SE = standard error, CI = calving interval, GARR = gross annual recruitment rate.

Years	Gestation period of 12 months					Gestation period of 13 months					Gestation period of 14 months				
	APR	SE	CI	SE	GARR	APR	SE	CI	SE	GARR	APR	SE	CI	SE	GARR
1976-77	0.118	0.009	8.5	0.68	0.027	0.109	0.009	9.2	0.77	0.025	0.101	0.009	9.9	0.87	0.023
1978-79	0.182	0.035	5.5	1.06	0.042	0.168	0.034	5.9	1.21	0.039	0.156	0.033	6.4	1.36	0.036
1980-81	0.250	0.027	4.0	0.43	0.057	0.231	0.026	4.3	0.49	0.053	0.214	0.026	4.7	0.56	0.049
1982-83	0.231	0.016	4.3	0.30	0.053	0.213	0.016	4.7	0.35	0.049	0.197	0.015	5.0	0.39	0.045
1984-85	0.333	0.017	3.0	0.16	0.077	0.308	0.017	3.2	0.18	0.071	0.286	0.017	3.5	0.20	0.066
1986-87	0.231	0.016	4.3	0.30	0.053	0.213	0.016	4.7	0.35	0.049	0.198	0.015	5.0	0.39	0.045
1988-89	0.200	0.010	5.0	0.25	0.046	0.185	0.010	5.4	0.28	0.042	0.171	0.009	5.8	0.32	0.039
1990-91	0.250	0.013	4.0	0.22	0.057	0.231	0.013	4.3	0.25	0.053	0.214	0.013	4.7	0.28	0.049
Mean	0.222		4.5		0.051	0.205		4.9		0.047	0.190		5.3		0.044

Table 3.11. Intermediate schedule: reproductive parameters, based on a gestation period of 12 months ($T_0=1.0$), 13 months ($T_0 = 1.08$) and 14 months ($T_0 = 1.17$) for female manatees collected in the southeastern United States (1976-91). APR = annual pregnancy rate, SE = standard error, CI = calving interval, GARR = gross annual recruitment rate.

Years	Gestation period of 12 months					Gestation period of 13 months					Gestation period of 14 months				
	APR	SE	CI	SE	GARR	APR	SE	CI	SE	GARR	APR	SE	CI	SE	GARR
1976-77	0.167	0.010	6.0	0.37	0.038	0.154	0.010	6.5	0.42	0.035	0.143	0.010	7.0	0.48	0.033
1978-79	0.200	0.027	5.0	0.67	0.046	0.185	0.026	5.4	0.76	0.042	0.171	0.025	5.8	0.85	0.039
1980-81	0.333	0.022	3.0	0.20	0.077	0.308	0.022	3.2	0.23	0.071	0.286	0.021	3.5	0.26	0.066
1982-83	0.310	0.016	3.2	0.16	0.071	0.286	0.015	3.5	0.19	0.066	0.266	0.015	3.7	0.21	0.061
1984-85	0.419	0.016	2.4	0.09	0.096	0.387	0.016	2.6	0.10	0.089	0.359	0.015	2.8	0.12	0.083
1986-87	0.400	0.014	2.5	0.07	0.092	0.369	0.014	2.7	0.10	0.085	0.343	0.013	2.9	0.11	0.079
1988-89	0.352	0.009	2.8	0.07	0.081	0.345	0.009	3.1	0.08	0.075	0.301	0.009	3.1	0.09	0.069
1990-91	0.400	0.012	2.5	0.08	0.092	0.369	0.012	2.7	0.08	0.085	0.343	0.012	2.9	0.10	0.079
Mean	0.333		3.0		0.077	0.308		3.2		0.071	0.286		3.5		0.066

Table 3.12. Optimistic schedule: reproductive parameters, based on a gestation period of 12 months ($T_0=1.0$), 13 months ($T_0 = 1.08$) and 14 months ($T_0 = 1.17$) for female manatees collected in the southeastern United States (1976-91). APR = annual pregnancy rate, SE = standard error, CI = calving interval, GARR = gross annual recruitment rate.

Years	Gestation period of 12 months					Gestation period of 13 months					Gestation period of 14 months				
	APR	SE	CI	SE	GARR	APR	SE	CI	SE	GARR	APR	SE	CI	SE	GARR
1976-77	0.231	0.011	4.3	0.20	0.053	0.213	0.010	4.7	0.23	0.049	0.198	0.010	7.0	0.48	0.045
1978-79	0.200	0.027	5.0	0.67	0.046	0.185	0.026	5.4	0.76	0.042	0.171	0.025	5.8	0.85	0.039
1980-81	0.364	0.022	2.7	0.16	0.083	0.336	0.021	3.0	0.19	0.077	0.312	0.021	3.2	0.22	0.071
1982-83	0.375	0.015	2.7	0.11	0.086	0.346	0.015	2.9	0.12	0.080	0.321	0.014	3.1	0.14	0.074
1984-85	0.500	0.014	2.0	0.05	0.115	0.461	0.014	2.2	0.06	0.106	0.428	0.014	2.3	0.07	0.098
1986-87	0.432	0.013	2.3	0.07	0.099	0.399	0.013	2.5	0.08	0.092	0.371	0.013	2.7	0.09	0.085
1988-89	0.417	0.008	2.4	0.05	0.096	0.385	0.008	2.6	0.05	0.088	0.357	0.008	2.8	0.06	0.082
1990-91	0.478	0.011	2.1	0.05	0.110	0.441	0.011	2.3	0.05	0.101	0.410	0.011	2.4	0.06	0.094
Mean	0.394		2.5		0.090	0.363		2.7		0.083	0.337		3.0		0.078

Figure 3.1. Frequency distribution of estimated age classes of 511 female manatees recovered dead between April 1976 and December 1991, in the southeastern United States.

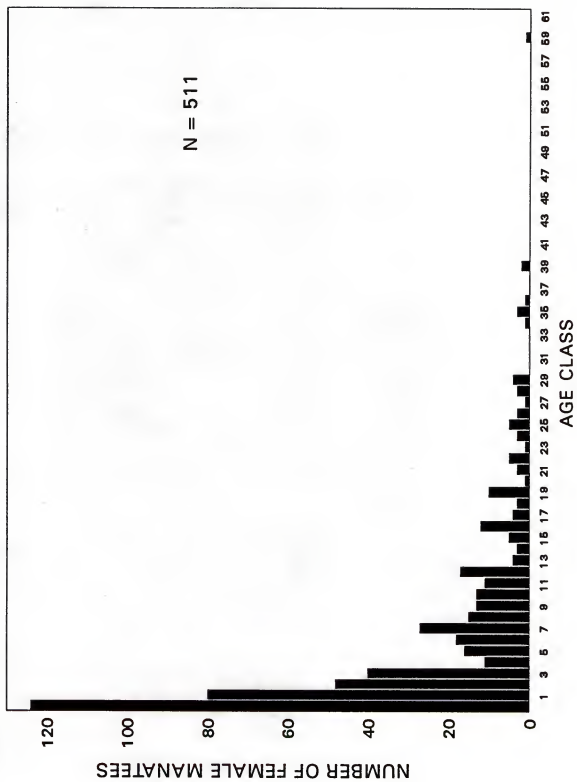


Figure 3.2. Reproductive status (pregnant, lactating, and resting) of mature female manatees in the carcass sample, in 2-year intervals (1976-91), for the southeastern United States (intermediate schedule).

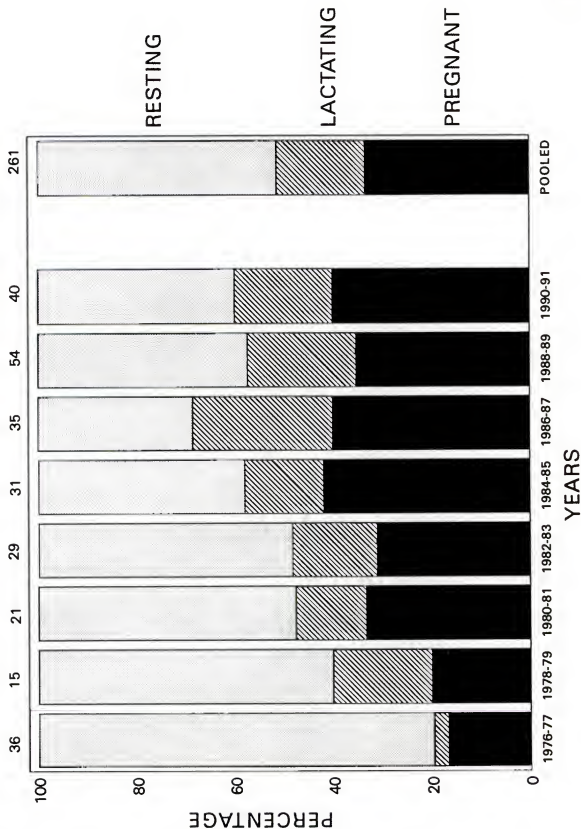


Figure 3.4. Length distribution of fetuses (gray squares) from dead female manatees, aborted fetuses (black squares), stillborn calves (gray circles), and calves that died soon after birth (black circles), collected in the southeastern United States (1976-91), by month.

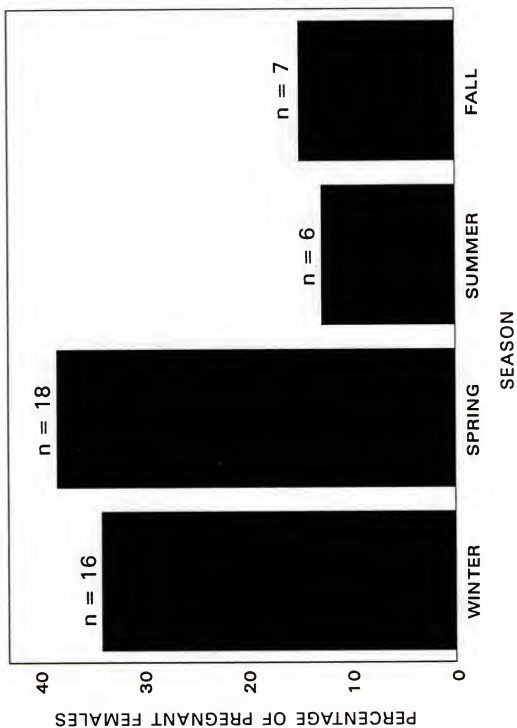


Figure 3.3. Incidence of pregnancy events by season (winter through fall) for female manatees collected in the southeastern United States (1976-91).

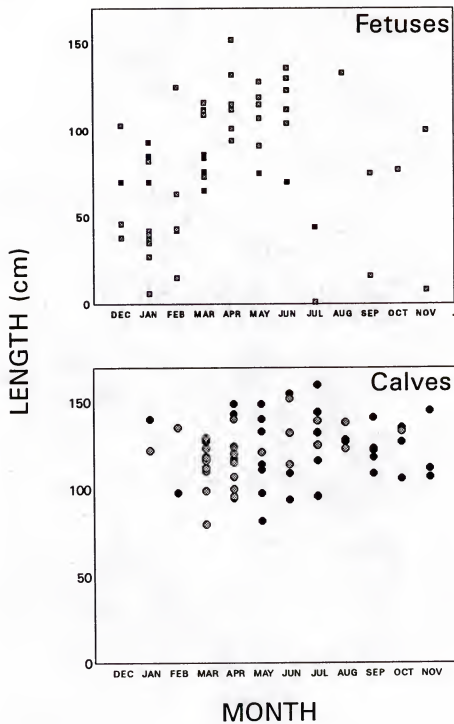
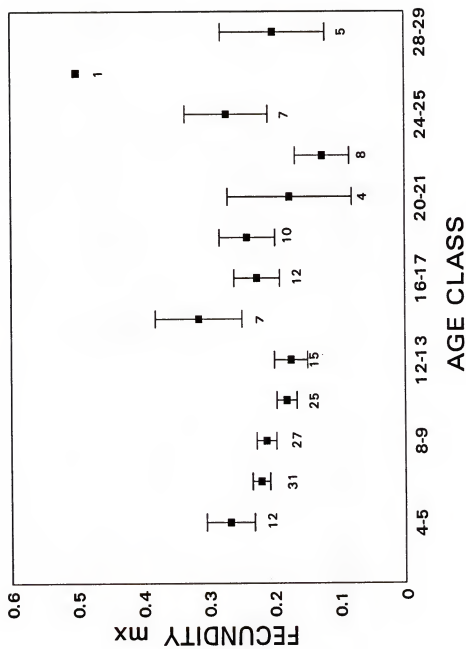


Figure 3.5. Female age-specific fecundity (female births/female/year) based on the sample of female manatee carcasses, by 2-year age-class intervals. Squares represent fecundity values, bars represent standard error, and numbers above bars represent sample size (intermediate schedule).



CHAPTER FOUR
AGE-RELATED ASPECTS OF MORTALITY
IN MANATEES

Introduction

Manatees' slow speed and dependence on seagrasses and shallow habitats renders them vulnerable to human-associated mortality. The main causes of mortality are collision with boats and barges, and undiagnosable factors in the perinatal period or during early calf dependency (Ackerman et al., 1992; O'Shea et al., 1985). Increases in the human population and the number of registered boats in Florida, and an apparent increase in manatee mortality over the years make it imperative that mortality rates be monitored. Chapman and Robson (1960) remarked that accurately estimating mortality rates was increasingly important for the study of dynamics of populations. Rate of increase of a population, reproductive values for each age class, and mean life expectancy at birth are useful parameters that require knowledge of age-specific mortality rates in order to be calculated (Caughley, 1966). Mortality is traditionally represented by a life table, which mimics the life history of a hypothetical group being depleted by deaths. The objective of this chapter was to analyze age-specific

mortality in Florida manatees and to build a life table for the population.

Materials and Methods

Data Collection and Determination of Cause of Death

Information on mortality causes and death categories was obtained through the carcass salvage network. Samples consisted of carcasses collected in the southeastern United States between 1976 and 1991. Although the network was started in 1974, data were analyzed only for the period 1976-91, because carcass and data collection were not fully operational until late 1975 (Campbell and Irvine, 1981). Reported carcasses were recovered whole whenever possible. Sex was determined and measurements taken according to standard procedures (Bonde et al., 1983). Depending on the degree of decomposition, a necropsy was performed to determine cause of death (see Table 4.1). I have departed somewhat from the death categories presented in Beck et al. (1981), Bonde et al. (1983), and Irvine et al. (1981). For example, "cold stress" and "red tide" are at times analyzed separately from the broader category "other natural", to allow discussion of these topics. Cases in which the necropsy was inconclusive or not conducted were termed "undetermined."

Specimens were aged based on the growth-layer groups in the tympano-periotic bone (see Chapter 2), and mortality rates were calculated from the age distribution of deaths.

Data Analyses

Because of the non-normal distribution of ages at death (see Fig. 2.34), I used non-parametric procedures to analyze the data. I tested hypotheses concerning differences in "age class" between sexes and coasts using the NPar1Way procedure (SAS Institute Inc., 1987). This procedure performs analyses of variance on ranks, using Kruskal-Wallis tests of whether the distribution of the variable has the same location parameter across the groups. To analyze differences among years, allowing for the influence of winter on mortality, I re-coded years as April of a calendar year through March of the following calendar year, to include full winter seasons. I tested for differences in the distribution of age of animals dying among years with analysis of variance through the GLM (general linear models) procedure, using the original data, as well as ranked data. Multiple pairwise comparisons were done for the entire data set, and a contrast statement was used to specifically test differences between years of cold winters (1976-77, 1980-81, 1984-85, 1989-90) versus ordinary winters.

The numbers of males and females recovered in different categories (fetuses, calves, subadults, and adult animals)

were compared by means of χ^2 goodness-of-fit tests (SAS Institute Inc., 1987).

Survivorship curves of males and females were compared through a log-rank test (Peto et al., 1977) on estimates calculated from the life table. The method consists of constructing a composite of the male and female life tables, calculating the expected number of deaths per age class for each sex, and calculating a χ^2 value by the standard formula.

The distribution of ages during three periods (1976-80, 1981-85, and 1986-91) was analyzed through a one-way ANOVA on ranks using the GLM procedure.

Life Table Analysis

I considered the female segment only in building the life table because of tradition and ease of assessing female fecundity. Therefore, the survival statistics refer to females, and births represent daughter births. To obtain overall values it suffices to add a male segment with similar growth parameters. Approximately 56% of specimens of total length less or equal to 150 cm were not processed, either due to unavailability or because it had previously been determined that such lengths correspond to animals of age class 0. In comparison, only 37% of the total number of specimens ≥ 150 cm were not processed. Therefore, the

number of age class 0 specimens was corrected from 123 to 176.

Assumptions for the construction of the life table

The construction of a composite life table based on ages at death obtained from recovered carcasses required the following assumptions: (1) the specimens are correctly aged; (2) the population has a stable age distribution (i.e., the proportion of each age class remains constant over time); (3) the age distribution of the sample is representative of the entire population; and (4) there is no differential loss by age class through decomposition, scavenging, or sinking. A fifth and fundamental assumption normally invoked is that population growth $r = 0$ (Caughley, 1977). On the other hand, if r is known and different from 0, the frequency of deaths may be corrected by e^x to produce a multiple of a d_x schedule (Caughley, 1977).

The life table

I constructed a frequency distribution of ages-at-death and assumed population rate of increase was 0. I then calculated population parameters (age-specific mortality, age-specific survival, survivorship, and stable age distribution) according to Caughley (1977), Lanciani (1987) and Mertz (1970).

Definitions of life table parameters. The subscript x represents age class. The value f_x represents the number of recovered female carcasses of a age class (original data).

Age class 39 represented 99.8% of the total sample, followed by an interval of 20 age classes until the last (age class 59). As no data on senescence are available, and in order to accomodate the decline accompanying it (Eberhardt, 1985) I truncated the distribution at age class 39, very similar to the value of 40 suggested by Eberhardt and DeMaster (1992). All columns in a life table can be calculated from each other.

The probability of dying in each age interval ($x, x+1$) is given by

$$d_x = \frac{f_x}{\sum f_x}$$

The probability at birth of surviving to age x (l_x , or survivorship function, or age-specific survivorship) is given by

$$1 - \sum_0^{x-1} d_x$$

Survivorship of the first age class (l_0) equals 1, by definition. Annual adult survivorship, assumed constant, was calculated by the "catch curve" method developed for fisheries by Chapman and Robson (1960) and Robson and Chapman (1961), where

$$s = \frac{T}{T+n-1}$$

provides a minimum-variance unbiased estimator of survivorship. In this formula, $n = N_0 + N_1 + N_2 + \dots + N_k$ where the N_i represent the number of individuals recovered in a particular age class in a segment of the age structure, or in my case

$$n = \sum_{x=0}^{\infty} D_x$$

and $T = N_1 + 2N_2 + 3N_3 + \dots + kN_k$ or

$$T = \sum_{x=0}^{\infty} xD_x$$

The calculation further requires that the first age included in the calculation be re-coded as age 0. The variance of this statistic may be calculated as (Robson and Chapman, 1961)

$$V(\hat{s}) = \frac{T}{n+T+1} \left[\frac{T}{n+T+1} - \frac{T-1}{n+T+2} \right]$$

Age-specific mortality q_x is the proportion of females dying in each age class

$$q_x = \frac{d_x}{l_x}$$

its complement is the age-specific survival rate (p_x).

The value for m_x (age-specific fecundity) was obtained from the number of pregnant and mature females from the carcass sample under the intermediate schedule of reproduction (see Chapter 3).

The product $l_x m_x$ (age-schedule of births and deaths) summarizes the two life-history functions (l_x and m_x), and therefore the life history of each member of the population. The rate of natural increase or Malthusian parameter is r in the differential equation $dN/dt = rN$, or $N_t = N_0 e^{rt}$; r is also the difference between the instantaneous birth rate (b) and death rate (d) in the population ($r = b - d$). Rate of increase was assumed 0 in this study. Instantaneous birth rate was calculated by (Birch, 1948)

$$\frac{1}{b} = \sum e^{-rx} l_x$$

If the l_x and m_x schedules remain constant, then the population will eventually reach the "stable" age distribution. The stable age distribution was calculated as (Mertz, 1970)

$$C_x = \frac{e^{-rx} l_x}{\sum_{y=0}^{\infty} e^{-ry} l_x}$$

or

$$C_x = be^{-rx} l_x$$

where the age structure was expressed as proportions, i.e., the sum of the C_x is unity.

Results

Age Distribution and Mortality

Based on the sample with estimated ages, the "boat/barge" category included most of the specimens where cause-of-death was known, followed by "dependent calf". Each of the other causes represented $\leq 10\%$ of the total (Table 4.2). The value for "cold stress" would be higher if cold stress cases earlier in the program had not been designated as undetermined. Seventy-two percent (680/864) of documented non-dependent calf deaths (where cause of death could be determined) were of anthropogenic origin.

Virtually half of the carcasses analyzed belonged to age classes 0, 1, and 2 (Table 4.3). The mean age in the total sample of aged carcasses ($N = 1,173$) was low (5.7 years, $SE = 0.22$). Age distributions of males and females

did not differ ($\chi^2 = 3.2135$, $df = 1$, $P = 0.0730$); age distributions between manatees from the two coasts differed slightly ($\chi^2 = 3.9465$, $df = 1$, $P = 0.0469$; Table 4.4), with mean ages lower on the west coast of Florida. It was not possible to detect any significant difference between age distributions from 1976-77 through 1990-91 with the untransformed data ($F = 1.25$, $df = 14$, $P = 0.2325$). Age distributions differed among individual years (April through March, Table 4.4) for ranked values ($F = 2.18$, $df = 14$, $P = 0.0070$). Two pairwise comparisons were significantly different at the 0.05 level: 1982-83 vs. 1986-87 and 1983-84 vs. 1986-87. However I cannot find an obvious explanation for these results. I was unable to detect a trend among years when contrasting years with cold winters versus normal winters ($F = 2.07$, $df = 2$, $P = 0.1506$).

Causes of Mortality

Age classes were not distributed equally among cause-of-death categories ($\chi^2 = 344.81$, $df = 6$, $P = 0.0001$; Table 4.5, Figs. 4.2a, b), with mean ages lower in the dependent calf and cold stress categories (Table 4.6, Fig. 4.1).

Early-life mortality

A large number of manatees died during the first year of life: 26% of all deaths were estimated at age class 0 (between 0 and 1 year of age), and 16% at age class 1 (Table 4.3). Most of those deaths were classified as "dependent

calf". With one exception, all animals in the dependent calf category (which by definition is restricted to very young calves) were estimated at age class 0 (Table 4.5). Boat strikes only represented 12% of the total deaths of this segment.

Subadult mortality

Cold stress associated with low winter temperatures was the most prominent natural mortality agent among subadults (age classes 2 and 3; Table 4.5). Mortality associated with cold weather during the winter 1976-77 was classified as "undetermined" because the relation of cold weather to deaths during those periods was uncertain (Irvine et al., 1981). Seventy-eight of the 94 cold-related deaths occurred on the east coast, a value significantly different from the west coast ($\chi^2 = 21.486$, $df = 1$, $P = 0.0$).

Impacts with watercraft also represented a large proportion of deaths in age classes 2 and 3. In fact, the proportion of animals killed by watercraft in those 2 age classes did not differ from the proportion of adults affected by the same cause ($\chi^2 = 0.428$, $df = 2$, $P = 0.807$).

Adult mortality

Except for cold stress and dependent calf mortality, all other cause-of-death categories seemed to include individuals indiscriminately across the age range (see Fig. 4.1 and Tables 4.5 and 4.6). Death due to collision with boats and barges was the category with the greatest numbers

of deaths among adults. The red tide category represented one isolated episode, so sample size is small ($n = 26$). Red tide may have affected animals through ingestion of ascidians (O'Shea et al., 1991), and age class 0 appeared to be the least affected, since those individuals were mostly nursing and eating smaller amounts of solid food.

Sex Ratio

Sex ratio among the 4 categories analyzed (unborn fetuses, stillborn calves, subadults, and adults) did not differ from 1:1, and differed slightly from 1:1 in dependent calves, due to a higher proportion of males in this category (Table 4.7).

Survivorship

Following a high death rate among the very young, the survival rate progressively increased up to age class 4, after which it remained approximately constant throughout life, at $89.6\% \pm 0.014$. In semi-logarithmic scale, a constant survivorship value is shown by a straight line ($\ln y = -0.1334 - 0.177x$, $r^2 = 0.9779$). Although this scale magnifies the least accurate portions of the curve (older age classes with small samples), showing flat regions separated by steps, the survivorship plot demonstrates the constancy in adult survival from age class 4 to at least 39 (Fig. 4.3).

Despite a tendency for greater mortality in males than females among many mammals (Dunbar, 1986; Ohsumi, 1979; Ralls et al., 1980; Trivers, 1972), the comparison of male and female survivorship curves did not differ significantly ($\chi^2 = 3.37$, $df = 1$, $P > 0.05$).

Although the columns in the life table (l_x , p_x , q_x , d_x) contain the same information, they provide information on mortality from different angles (Fig. 4.4). The survival curve graphically shows the proportional strength of each age class in the population, illustrating the structure of the population. The mortality curve illustrates the pattern of losses of a population (Quick, 1963). Caughley (1977) maintains that q_x is the least affected by sampling bias and provides the most direct projection of the mortality pattern. Normally, mortality (q_x) after birth is quite high in mammals, and may decrease steadily until old age, when it may increase again (Caughley, 1966). The mortality value is independent of frequencies of younger age classes and shows mortality directly, whereas the survivorship schedule only shows mortality by the slope of the curve.

Life Table Analysis

Survival of female calves in age classes 0 and 1 was calculated as 0.685 and 0.794, respectively. Survival of subadults (age classes 2 and 3) was 0.842 and 0.844. Adult survival was variable but on average was greater than

subadult survival (Table 4.8). The mean adult survival rate was calculated as 0.896 ± 0.014 . Survivorship estimates for populations on the Atlantic and Gulf coasts of Florida did not differ (logrank test, $\chi^2 = 0.21$, $df = 1$, $P > 0.5$).

The instantaneous birth rate was calculated as 0.15632. Fecundity, survivorship, and net reproductive rate are intimately related, the reproductive rate depending not only on fecundity but also on the fate of the individuals at various age classes (Fig. 4.5).

The stable age distribution of manatees (Fig. 4.6) indicates that the population is composed of approximately 16% individuals in the zero age class, 26% subadults, and 58% adults (age classes 4-39).

Discussion and Conclusions

Early-Life Mortality: Dependent Calves

Dependent-calf mortality affected young animals in the first year of life (age previously estimated based on length and gross anatomical features), without further detailing of the proximate agent causing death. The definition of dependent calf as less than or equal to 150 cm total length underestimates true perinatal mortality, because some calves are larger than that at birth. Mortality was high among these young animals (age class 0), which constituted 26% of the total carcass sample. Animals at age class 1 also were

subject to relatively high mortality (22%). Weaning was a major event between 1 and 2 years of age, and animals in this age class lost the benefits of the mother's care and experience (O'Shea et al., 1985). High mortality during the juvenile phase is typical among mammals (Buss, 1990; Caughley, 1966; Spinage, 1970).

The proportion of pregnant females increased over the last 15 years (see Chapter 3), and the percentage of perinatal deaths in the carcass sample has also increased by 12%/year since 1976 (Ackerman et al., 1992). The carcass sample includes a large proportion of young animals (overall mean = 5.7 years). Young manatee mothers may be more prone to miscarriages resulting in abortions or stillbirths, as occurs in adolescent specimens of other species (Martin, 1981; Newson, 1966; Sowls, 1966). In addition, if younger females are more likely to abandon their calves, more newborn animals up to year 1 might be expected to die. The very close association frequently observed between a cow-calf pair indicates that the presence of the mother is essential for calf survival. Although calves may start nibbling on plants at an early age (Husar, 1977), they greatly depend on the high-quality nutrition provided by the mother's milk to grow quickly. The survival of orphaned or abandoned young calves therefore seems highly unlikely unless foster parenthood occurs at a higher-than-expected level.

Subadult Mortality: Cold Stress

Cold temperatures contributed to the death of manatees at a range of ages. Nevertheless, the average age affected was 2.7, reflecting a greater effect on age classes 1 through 3. In addition to having recently been weaned, younger individuals had larger surface area/volume ratios. Smaller animals probably lost proportionately more heat than the larger, older ones and, therefore, were stressed more by cold weather. Other factors involved in subadult mortality may have included nutritional stress during cold weather (many of these individuals had depleted fat reserves) or unfamiliarity with traditional migration patterns and locations of warm water refugia (O'Shea et al., 1985).

Susceptibility to low winter temperatures is evidenced by a historic vulnerability to low temperatures, a low metabolic rate, and a limited capacity for heat production (Buergelt et al., 1984; Irvine, 1983). Manatee die-offs have occurred historically under conditions of extreme winter temperatures (Bangs, 1895; Cahn, 1940; Layne, 1965; Moore, 1956). The very cold winters of 1976-77 (Campbell and Irvine, 1981), 1980-81 (Bonde, 1982), 1984-85, and 1989-90 were associated with high numbers of dead manatees (Ackerman et al., 1992). A good discussion on cold-related mortality in recent years may be found in O'Shea et al. (1985).

During the warm months, manatees disperse throughout Florida, but during winter they aggregate in natural or industrial warm-water sites. The latter option has changed manatees' traditional southerly movements during the winter. In this way manatees have expanded their historical winter range limits but have become susceptible to increased cold-induced mortality during unusually severe winters (Campbell and Irvine, 1981; O'Shea et al., 1985). The east coast has more industrial warm-water sites than the west coast. Perhaps winter mortality was more prominent on the east coast because a greater number of east coast individuals are dependent on artificial sources of warm-water that failed to provide adequate protection in unusually cold weather. Aerial surveys of power plants after cold fronts during the winter of 1991-92 produced an average of 352 manatees in 4 power plants on the east coast compared to 108 manatees in one of the two power plants frequently used by manatees on the west coast (Reynolds, 1992).

The similarity in the proportion of manatees killed by boats from age class 2 onwards indicates that age has no bearing on the probability of manatees being hit by boats.

Adult Mortality Due to Collisions with Boats

By far the most important mortality factor affecting adult manatees is collision with watercraft, explained by the vulnerability of manatees in shallow waterways (see

Beck et al., 1981). According to Ackerman et al. (1992), incidence of this type of mortality has increased 10% yearly since 1976. Mortality due to accidents with watercraft affected the entire range of age classes, from newborn to the oldest specimen (AC ~59).

Eberhardt and Siniff (1977) pointed out that high adult survival is crucial for maintenance of a positive rate of increase in large mammals. The long potential life span of manatees indicates a naturally low adult mortality rate, necessary to counterbalance the relatively slow reproductive rate. This study has produced statewide adult survival rate estimates of 0.896 ± 0.014 . These values are comparable to survivorship values obtained from studies based on mark-recapture through scar-catalogued manatees (O'Shea and Langtimm, in manuscript) for the Atlantic coast (0.877, 0.848-0.0906 95% confidence interval, 1984-91). However, they are lower than those calculated for Crystal River (0.962, 0.943-0.981 95% confidence interval, 1977-78 to 1990-91) and Blue Spring (0.948, 0.908-0.988 95% confidence interval, 1977-78 to 1990-91). Both Crystal River and Blue Spring include sanctuaries for manatees where they are granted full protection in winter, and much of the year-round habitat is less developed than the Atlantic coast. Manatees travelling among sites along the Atlantic coast through the Intracoastal Waterway are likely to be considerably more susceptible to fatal encounters with

watercraft, due to higher boat traffic and development. Because a much larger proportion of the carcass sample is from the Atlantic coast and west coast areas more urbanized than Blue Spring or Crystal River, this may explain the similar rates obtained through mark-recapture studies of the Atlantic coast and the discrepancies between my statewide survival estimates and estimates limited to the Crystal River and Blue Spring areas.

Sex Ratio

The theory of sex allocation states that parents should invest equally in male and female offspring, which results in a 50:50 sex ratio (Fisher, 1930). A population's sex ratio may change due to an unbalanced sex ratio at birth (Seal and Lacy, 1990) or due to differential mortality with age associated with each sex (Ralls et al., 1980) such as vulnerability to hunting, predation, or competition.

Manatees comply with the general pattern of equal sex ratios at birth common to most vertebrates (Caughley, 1977; Emmel, 1976). Except for a marginally significant difference between males and females in the dependent-calf category, no imbalance in the sex ratio throughout life was evident from the manatee carcass sample. Because the level of significance was borderline and significant differences were not observed at any other stage of life, the slight

preponderance of males in the dependent-calf category may be ignored.

A departure from a 50:50 ratio (principally through differential mortality) could affect the demography of a species, depending on the mating habits (Shaw, 1985) and any change in the ratio may influence population growth. Because manatee females may mate with several males (Hartman, 1979) and males tend to move considerably more than females (O'Shea and Kochman, 1990), the number of males probably does not limit the reproductive success of females, and the number of males makes little difference in simple population models (Stearns, 1992). Therefore, the number and survivorship of breeding-age females is more critical than that of adult males to maintenance of the population, and problems are likely to occur only under extremely skewed situations.

Life Table Analyses

The pattern of changing mortality rates with age is best expressed in the form of a life table (Caughley 1966), which identifies age classes most affected by mortality. Deevey (1947) pioneered the quantification of mortality and construction of life tables for animal populations, using Murie's (1944) data on a population of Dall's sheep (*Ovis dalli*) in Mt. McKinley National Park. Life tables for

marine mammals have been constructed in only a few cases (Hewer, 1964; Kenyon et al., 1954; Lander, 1982).

Very rarely are real cohorts studied, especially in the case of large mammals, due to the long time and capital commitment required to gather sufficient data. "Current", or "vertical" (time-specific), life tables are not as accurate as "cohort" or "horizontal" life tables, that follow a cohort throughout life (age-specific), but the latter are usually most feasible for short-lived species. In the case of a long-lived species like the manatee, other techniques such as mark-recapture and censuses may provide more reliable estimates of survival and population growth than life tables, but the former have not been developed sufficiently at this point, and aerial surveys are still experimental. Current and cohort life tables are identical only when the age-specific mortality is exactly the same from year to year. The survivorship values presented here were computed indirectly from age-specific death rates through a composite life table.

The life-table format has been questioned in the literature, and in a review of life tables, Caughley (1966) and Ralls et al. (1980) pointed at a number of works that did not meet one or more of the basic assumptions. Seber (1982) pointed at confusion derived from the improper use of life tables with time-specific data and suggested the use of Chapman and Robson (1960) estimates as better approaches in

calculating survivorship. Caughley and Birch (1971) indicated the circularity of assuming $r = 0$ in building a life table from ages-at-death, in which the rate of increase cannot be calculated. Van Sickle (1988) demonstrated that such calculation always results in a value of or very close to 0, and is totally due to sampling variation. Tait and Bunnell (1980) offered a method of calculating growth rate and survivorship by combining ages at death with a fecundity schedule, but they concluded that growth rate estimates would be unreliable due to sampling errors. Van Sickle et al. (1987) described a method using the same data that is robust to sampling errors and flexible to use with birth-pulse or birth-flow population data. It is recommended that such approach be attempted with the manatee carcass data to supplement or verify results obtained in my study.

On the assumptions

Conclusions from a life table will be unreliable if the assumptions on which the table is based are incorrect. One point that may be questioned is the accuracy of age determination. All ageing methods contain some bias or inaccuracy, and the final age distribution may differ somewhat from the truth (Chapter 2). Nonetheless, the method I used here was validated with known-age animals, and probably represents a very good approximation of reality. This is particularly true for younger animals, which it turns out were the bulk of the sample.

Another question is whether the carcass sample provides an unbiased estimate of the age structure of the population. One aspect of this problem lies in the intensity of sampling. Given the scale of the carcass salvage program and the fact that most of the coast is inhabited by people, I assumed that the number of unrecovered or unreported carcasses is small and that the sample is a very large proportion of the total dying. If samples are missed in less-intensely covered areas, such as portions of the west coast, missed observations are likely to be proportional to the number of deaths across age classes. Several studies point to higher perishability or lower conspicuousness of small carcasses due to predation or degradation (Bourlière and Verschuren, 1960; Laws, 1966). Young manatee carcasses are more likely to be missed and decompose faster than larger animals, so age class zero is likely to be biased low. The early portion of the survivorship curve may indicate some underrepresentation of small specimens. However, the 31% age-class-zero mortality is comparable to the value of 33% obtained in the field at Crystal River by Rathbun et al. (1992), based on females assessed as pregnant in one winter and returning the following season without an accompanying calf.

Probably most difficult to defend is the assumption of a stable age distribution. This implies that age-specific birth and death rates remained constant for a long period

before the age distribution was sampled (Lopez, 1961). The most accurate way to determine stability is through direct observations and calculation of the proportion of each age class, but manatee censuses are still problematic. A good indication of stability is given if the proportion of 1 or 2 age classes remained similar for a few years. The proportion of calves in aerial and field surveys may provide an independent indication of stability. Various studies (Powell, 1981; Rathbun et al., 1990; Reynolds, 1992) have shown the proportion of calves in the population to be around 10% (Table 4.9). Even though that only represents 1 age class, it has remained roughly constant over the years. Lotka (1907), Sharpe and Lotka (1911), and Caughley and Birch (1971) showed that a stable age distribution would be assumed if the age-specific fecundity (m_x) and survivorship (l_x) were constant over a number of years or several generations. Although departures from the stable age distribution may result in oscillations as the age structure stabilizes, such departures are rarely substantial for large mammals (Eberhardt, 1985). Stearns (1992) points out that a population may not actually be growing at the calculated r if it is not in stable distribution, but that would be the growth rate towards which the population is converging. The same author clarifies that rarely natural populations are in stable age distribution, but when they are not they are usually returning to one. Under fixed schedules of l_x and r

a population's age distribution will converge towards stability, independent of rate of increase (Caughley, 1977). It is likely that numerous demographic transitions affected the 15-year sample on which the manatee life table is based (i.e., increasing watercraft-related mortality, increased reproductive rate), although the pattern of fecundity and survivorship may have remained constant. Caughley (1977) commented that even if fluctuations in r occurred, if the time frame over which those occurred was much smaller than the time over which the sample was collected, the method should provide a "reasonably accurate life table". Despite random variations in fecundity and survival, many populations of large mammals will tend to a stable age distribution, and large departures from that level are unlikely except under very severe, rare events (Goodman, 1981). Evaluation of stationarity requires the examination of a chronological sequence of age distributions (Seber, 1973). I also examined the stable age distribution at death (the counterpart of the stable age distribution) by partitioning the data in 3 periods of similar length (1976-80, 1981-85, and 1986-91), and covering sample sizes minimally adequate to allow life table analysis (Caughley, 1977). The similarity in the distribution of ages at death in the 3 time periods ($F = 1.97$, $df = 2$, $P = 0.1393$) generally supports the assumption of a stable distribution (Table 4.10).

Caughley (1977) warned against the use of age-at-death data unless the assumptions are at least approximated. The assumptions appear to be reasonably satisfied by the carcass data, and their use is the only currently available means of obtaining a life table. Modeling based on these estimates should be useful, at the very least to show the importance of each parameter to the dynamics of the population, even if biases or inaccuracies are discovered later.

Life table parameters

In most vertebrate populations, both fecundity and mortality rates change with age. The analyses of age-specific fecundity in manatees indicate that all years beyond age class 4 are equal in terms of reproduction (see Chapter 3; Fig. 3.5). Age-specific survival also stabilizes once animals reach adulthood, following a high mortality rate in the first years. The survivorship for age-class 0 obtained from the life table conforms with field observations (Rathbun et al., in manuscript). Subadult survival is influenced by processes such as weaning, growing, and learning to feed and move in a new environment. Survival rates of adult mammals are much less variable than prepubertal rates; their yearly variation contributes little to fluctuations in rate of increase (Caughley, 1977), although their magnitude may be critical. The calculated average adult survival rate resembles estimates based on sight-resight data from free-ranging manatees (O'Shea and

Langtimm, in manuscript) as well as rates for several species of pinnipeds (Chapman, 1973; Eberhardt, 1981; Eberhardt and Siniff, 1977).

A crude estimate of r_m (maximum, intrinsic rate of increase) can be obtained by multiplying the maximum litter size by the maximum proportion of females likely to breed under ideal conditions ($0.4 \text{ offspring/year} * 0.23 \text{ reproducing females} = 9.2\%$). However, this does not account for mortality, or variability in the rates, and is quite unlikely to be accomplished in the wild. In general the maximum population growth for large mammals is very modest, usually below 10% (Allen, 1981; Barlow, 1986; Eberhardt, 1981; Eberhardt and Siniff, 1977; Laws, 1979; Marsh et al., 1984b; Reilly and Barlow, 1986; Summers, 1978). More realistically, population growth rates should fluctuate around zero, and only rarely could a population experience long, uninterrupted periods of increase or decline (Stearns, 1976), although such situation is not so uncommon nowadays due to human influences. The calculated birth rate (0.156) resembles the gross birth rate of 0.13 obtained from a field study by Rathbun et al. (1992).

Packard (1985b), in some preliminary simulations, suggested a range of possible values for growth rates of manatees. Under high parameter values, growth could vary between 2 and 7%, but with less favorable parameters, growth could be as low as -2.5% a year. Marsh et al. (1984b) found

a maximum rate of increase for the related dugong of the Indo-Pacific on the order of 5%. The method used in the present study does not allow calculation of r , so a rate of increase of 0 was assumed. Similarity between carcass-based estimates of population parameters and estimates derived from free-ranging animals (O'Shea and Langtimm, in manuscript; Rathbun et al., in manuscript) lend support to its use. Assuming the manatee population in the southeastern United States is subject to the calculated age-specific schedules of fecundity and mortality (constant in time), lives in a stable environment without food or space limitations, and is not subject to immigration or emigration, then if N is the number of manatees in the population, $dN/dt=rN$, and population numbers would be stable.

The age structure of endangered species in general, and of manatees in particular, is an obvious concern: the percentage of calves calculated from the stable age distribution falls within the range of values obtained from field studies and is intimately related to birth rate. Estimates for the proportion of calves in the population, based on aerial surveys (1977-91), ranged from 6.6 to 13.3. Irvine and Campbell (1978) remarked that their values (9.6%) were "probably conservative". Surveys conducted on power plants following cold fronts by Reynolds (1992) produced values of 6.6 to 12.8%, with an average of 9.68. Values for

a population in Crystal River ranged from 10.2% to 22.9% (1967-78) (Powell, 1981), and averaged 13.5% for 15 winter seasons (1977-78/1990-91, range 9.7-17.9) (Rathbun et al., in manuscript). Some of the discrepancy between the aerial and field estimates and the numbers from the carcass sample may be due to the season of survey and the definition of "calf". Most of the field and aerial counts were done during the winter, whereas most births seem to occur in spring-summer. Powell (1981) used suckling and attendance with a female as a criterion. Reynolds (1992) classified calves as "animals less than half the length of a closely-associated animal".

A large proportion of the reproduction comes from females in the interval AC 4-12, when fecundity is constant and survivorship is high (Fig. 4.5). Because of low fecundity, a long potential life span is generally required to maintain a high net reproductive rate in large mammals.

Longevity, reproduction and survival comprise a suite of co-evolved characteristics reflecting the species' response to its environment (Cole, 1954). Manatee life-history traits include high longevity, slow reproduction, and high adult survival. Individual manatees have also been shown to adapt well, learn to use sanctuaries, and survive boat encounters. The population may be able to absorb small, short-term oscillations in the rate of growth, but it could not quickly recoup from a major catastrophe. The

development trend in Florida presages even higher boat-caused mortality, posing a threat with which the species may not be able to cope. The assumed stability indicates very little margin for adjusting to increased mortality.

Furthermore, there are still unknowns in demographic terms: we do not know how various factors may affect reproduction. It is critical to understand if pregnancy rates are a density-dependent mechanism with which the population deals with mortality.

Longitudinal studies of living manatees now being carried out at Crystal River and Blue Spring (U.S. Fish and Wildlife Service, unpublished data) should be continued to refine estimates, account for regional differences, and provide a monitoring system of the manatee population. Because the carcass sample contains little representation from these protected areas (i.e., Crystal River and Blue Spring), it is not unreasonable to think that growth rate will show a more positive value when data from those areas is factored in. However, given the narrow range of possible growth around an equilibrium value, factors that would serve to increase adult survival and enhance reproduction should help to increase population growth and should be promoted. Measures to reduce mortality and increase habitat quality are particularly important. A photogrammetry study as suggested by the working group on age structure during the manatee workshop (O'Shea et al., 1992) and planned to be

carried out in winter 1992-93 may provide insight into the age structure of the wild populations of different areas.

Survivorship Analysis

The information on survivorship may be presented graphically as a survivorship curve, where the fraction of animals born and surviving to a given age is plotted against age. Changes in mortality can be directly observed from the survivorship curve, where the slope at any point is proportional to the instantaneous rate of mortality at that age (Caughley, 1966). Although the carcass data do not provide clear support, because of the small number of very old animals, it is reasonable to suppose that manatees follow the typical "U-shaped" mortality curve of mammals.

Different age classes normally present different death rates. Pearl (1928) and Pearl and Miner (1935) described the extreme shapes that survivorship curves could assume. No population has a survivorship curve exactly like the ideal ones described, and Pearl (1940) pointed out that these are conceptual models, not necessarily reflecting the empirical reality.

Mortality rates among mammals tend to be generally higher among the juvenile and very old age classes, while animals in their middle years suffer the lowest mortality rates (Caughley, 1966). Juvenile mortality rates in mammals tend to vary much more than adult mortality, which

contributes less to fluctuations in the rate of increase (Caughley, 1977; Charnov, 1986). Siler (1979) described the same phases in terms of competing risks: a decreasing hazard due to adjustment of the animal to its environment; a constant hazard, reflecting those factors to which the animal does not adjust; and an increasing hazard due to senescence. Caughley (1966) presented data on the similarity between mortality patterns among different taxonomic and ecological groups. Deevey's survivorship curve (1947: fig. 2) for the Dall's sheep and Mech's (1966) life table for moose show high early mortality, followed by a near plateau of constant high survival until physiological senescence begins, and finally a rapid decline in survival. The main mortality factor in those two species is predation by wolves, which are more likely to prey upon the weak and infirm. A similar survival curve was derived for large African ungulates (Spinage, 1970, 1972) and the few examples of marine mammals for which adequate data exist to develop life tables (Chapman, 1964; de la Mare, 1986).

Eberhardt and Siniff (1977) point out that the rapid initial drop/flat phase/accelerating drop pattern applies to most wild populations "at least in the absence of major human influences". Emigration or exploitation may change the shape of survivorship curves. The general shape of the manatee survivorship curve is consistent with the type expected in a long-lived mammal, following the pattern of a

higher death probability at very young ages followed by a constant, relatively low chance of death operating over the entire life table. However, it differs from most other large mammal curves in showing no apparent plateau during middle ages, but a steeper gradient than normally seen. Survival curves for waterbuck (Spinage, 1970) that follow the mammalian standard become steeper when a higher proportion is taken out of each age class, by emigration. Squirrels in a predator-free area but subject to hunting activity show a diagonal-type survivorship curve (Mosby, 1969). Hunting in grizzly bears increases the adult mortality rate (2-3 times higher than in unhunted situations), steepens the curve and removes the "kink" separating adults from subadults (Bunnell and Tait, 1981). A similar case was based on a sample of jaws of elephants dying due to natural causes in two parks in Uganda, showing an annual adult mortality of 4 and 5% (Laws, 1966). The survivorship curve in this case also does not have a plateau. The manatee curve is similar in shape but exacerbated by higher levels of mortality (>9%). Manatees are exposed to an added, constant kind of mortality for which there is no co-evolved defense. Unlike most prime-aged moose or sheep, which are able to escape wolf attacks, healthy adult manatees have no co-evolved defense mechanism against increased numbers of speeding watercraft sharing the waterways. The boat-caused mortality may produce a similar

effect in the manatee population as emigration and hunting do in the above examples, reducing survival rate.

Senescence

Although senescence is important in understanding life histories, few data on this topic are available for large vertebrates (Eberhardt, 1985; Eberhardt and Siniff, 1977). In the absence of human interference, and if survivorship through early maturity is quite high, one cannot neglect the demographic role of senescence (Eberhardt, 1985). Manatees fail to show a conspicuous final decline in survivorship towards the older classes. Senescence may not have a chance to manifest itself in manatees because anthropogenic causes cull animals before they reach that stage (only 1% of the total carcass sample was documented to be older than age class 29). In addition, if the population started to increase within the last 20 years, the number of senescent-age animals would not be expected to be large. Although a large proportion of the manatee population is composed of adults (58%) that survived the earlier hazards, only 1.5% are projected to be > 30 years of age. Even though the number of individuals in the later categories is small, at least one individual attained old age, close to what is believed to be physiological longevity based on studies of dugongs (Marsh, 1992), and manatees in captivity (see Chapter 2). Ecological longevity or the average length of

life observed under prevailing conditions in the
southeastern United States appears to be much shorter.

Table 4.1. Summary of factors considered for the determination of cause-of-death of Florida manatee carcasses, 1976-91 (adapted from Beck et al., 1981, Bonde et al., 1983, and Irvine et al., 1981).

Death category	Diagnostic characteristics
Boat/barge	Severe wounds, sometimes accompanied by broken bones. Massive internal damage and no other potential alternative causes probable; animal found in or near narrow channels known to be used by large barges.
Gate/canal	External scrapes and impressions, internal injuries, sometimes broken bones; recovery location in proximity to one of those structures.
Other human	Indications of death in association with human activities, such as entrapment in fishing nets, traps, or lines; ingestion of fishing gear or debris; gunshot wounds, indications of vandalism or poaching; entrapment in culverts or canals.
Dependent calf	Arbitrarily defined to include small calves ≤ 150 cm total length, which could not be placed in other categories; larger animals with signs of disease and congenital anomalies, or with obvious characteristics (umbilical appearance, digestive tract contents, lack of inflation of lungs, fetal folds).
Natural	Infectious or non-infectious disease, or natural catastrophe. Severe cold-weather-related death was identified by emaciation and general state of cachexia, reduced fat deposits, serous atrophy of fat, and sometimes empty digestive tract. Poisoning by biological toxins identified by pathologic findings and circumstantial evidence.

Table 4.2. Death categories affecting Florida manatees between 1976-91. These totals exclude cases where cause-of-death category was undetermined.

Cause of death	Frequency	Percent
Boat/barge	479	38.7
Dependent calf	337	27.2
Cold stress	124	10.0
Other natural	120	9.7
Gate/canal	83	6.7
Other human	58	4.7
Red tide	37	3.0

Table 4.3. Proportion of Florida manatee carcasses recovered in each age class, 1976-91 ($N = 1,173$).

Age class	Frequency	Proportion
0	305	26.0
1	192	16.4
2	107	9.1
3	75	6.4
4	35	3.0
5	39	3.3
6	46	3.9
7	49	4.2
8	40	3.4
9	26	2.2
10	33	2.8
11	21	1.8
12	24	2.0
13	21	1.8
14	16	1.4
15	8	0.7
16	20	1.7
17	9	0.8
18	10	0.9
19	14	1.2
20	7	0.6
21	9	0.8
22	8	0.7
23	1	0.1
24	11	0.9
25	11	0.9
26	6	0.5
27	6	0.5
28	6	0.5
29	7	0.6
30	1	0.1
34	1	0.1
35	3	0.3
36	1	0.1
37	2	0.2
39	2	0.2
59	1	0.1

Table 4.4. Statistics for the age-class parameters, compared by sex, coast, and year for Florida manatees, 1976-91. Years span April through March.

Variable	N	Range	Mean	SE
Sex				
Female	507	0 - 59	6.1	0.358
Male	563	0 - 37	5.2	0.295
Coast				
East	638	0 - 59	6.0	0.306
West	467	0 - 36	5.4	0.342
Year				
1976-77	25	0 - 29	8.2	1.646
1977-78	45	0 - 35	8.1	1.208
1978-79	36	0 - 27	7.6	1.274
1979-80	58	0 - 59	9.9	1.297
1980-81	58	0 - 37	7.1	1.297
1981-82	79	0 - 26	7.7	0.928
1982-83	54	0 - 28	6.9	0.868
1983-84	77	0 - 29	7.7	0.939
1984-85	69	0 - 28	6.5	0.874
1985-86	56	0 - 24	5.6	0.786
1986-87	48	0 - 29	6.3	0.752
1987-88	66	0 - 39	7.9	0.905
1988-89	69	0 - 36	8.0	0.978
1989-90	161	0 - 28	6.4	0.964
1990-91	100	0 - 37	7.8	0.503

Table 4.5. Incidence of mortality causes by age class, in Florida manatees, 1976-91.
Percentages of age class given in parentheses.

Cause of death	Age class				
	0	1	2	3	≥ 4
Dependent calf	157 (70.4)	1 (0.9)	0	0	0
Cold stress	16 (7.2)	33 (28.7)	16 (23.5)	8 (15.7)	21 (6.6)
Red tide	1 (0.4)	5 (4.3)	2 (2.9)	3 (5.9)	15 (4.7)
Other natural	15 (6.7)	21 (18.3)	10 (14.7)	7 (13.7)	37 (11.6)
Boat/barge	26 (11.7)	44 (38.3)	35 (51.5)	29 (56.9)	176 (55.3)
Gate/canal	1 (0.4)	8 (7.0)	4 (5.9)	3 (5.9)	42 (13.2)
Other human	7 (3.1)	3 (2.6)	1 (1.5)	1 (2.0)	27 (8.5)

Table 4.6. Summary statistics on the ages affected by each mortality cause in Florida manatees, 1976-91.

Cause of death	Age class			
	N	Range	Mean	s.e
Dependent calf	157	0 - 1	0.0	0.0
Cold stress	94	0 - 14	2.7	0.34
Other natural	88	0 - 27	5.7	0.73
Red tide	26	0 - 19	6.0	0.97
Boat/barge	310	0 - 59	8.0	0.49
Gate/canal	58	0 - 28	8.0	0.80
Other human	38	0 - 39	8.3	1.42

Table 4.7. Sex ratio for different age categories of manatees collected in the southeastern United States (1976-91) and chi-square tests for even distribution, adjusted for continuity (df = 1).

Category	Males	Females	Ratio	χ^2	P
Unborn fetuses	24	14	1.71	0.857	0.355
Stillborn or aborted calves	20	21	0.95	0.0	1.0
Dependent calves (age classes 0-1)	605	508	1.19	4.062	0.044
Subadults (age classes 2-3)	80	87	0.92	0.075	0.784
Young adults (age classes 4-19)	193	182	1.06	0.108	0.742
Medium adults (age classes 20-34)	32	30	1.07	0.00	1.000
Old adults (age classes 35-59)	2	7	0.28	0.542	0.462

Table 4.8. Life history parameters for female Florida manatees based on the life table approach, assuming stationarity ($r = 0$). Terms are further defined in the text under the heading "Definition of life table parameters".

Age x	Frequency of deaths f_x	Mortality d_x	Survivorship l_x	Mortality rate q_x	Survival rate p_x	Stable age distribution
0	176	0.314848	1	0.314848	0.685152	0.15632
1	79	0.141324	0.685152	0.206266	0.793734	0.107103
2	48	0.085868	0.543828	0.157895	0.842105	0.085011
3	40	0.071556	0.457961	0.15625	0.84375	0.071588
4	11	0.019678	0.386404	0.050926	0.949074	0.060403
5	16	0.028623	0.366726	0.078049	0.921951	0.057327
6	18	0.0322	0.338104	0.095238	0.904762	0.052852
7	27	0.048301	0.305903	0.157895	0.842105	0.047819
8	15	0.026834	0.257603	0.104167	0.895833	0.040268
9	13	0.023256	0.230769	0.100775	0.899225	0.036074
10	13	0.023256	0.207513	0.112069	0.887931	0.032438
11	10	0.017889	0.184258	0.097087	0.902913	0.028803
12	17	0.030411	0.166369	0.182796	0.817204	0.026007
13	4	0.007156	0.135957	0.052632	0.947368	0.021253
14	3	0.005367	0.128801	0.041667	0.958333	0.020134
15	5	0.008945	0.123435	0.072464	0.927536	0.019295
16	12	0.021467	0.11449	0.1875	0.8125	0.017897
17	4	0.007156	0.093023	0.076923	0.923077	0.014541
18	3	0.005367	0.085868	0.0625	0.9375	0.013423
19	10	0.017889	0.080501	0.222222	0.777778	0.012584
20	1	0.001789	0.062612	0.028571	0.971429	0.009787
21	3	0.005367	0.060823	0.088235	0.911765	0.009508
22	4	0.007156	0.055456	0.129032	0.870968	0.008669
23	1	0.001789	0.048301	0.037037	0.962963	0.00775
24	3	0.005367	0.046512	0.115385	0.884615	0.007271
25	4	0.008945	0.041145	0.217391	0.782609	0.006432
26	3	0.005367	0.0322	0.166667	0.833333	0.005034
27	1	0.001789	0.026834	0.066667	0.933333	0.004195
28	3	0.005367	0.025045	0.214286	0.785714	0.003915
29	4	0.007156	0.019678	0.363636	0.636364	0.003076
30	0	0	0.012522	0	1	0.001957
31	0	0	0.012522	0	1	0.001957
32	0	0	0.012522	0	1	0.001957
33	0	0	0.012522	0	1	0.001957
34	1	0.001789	0.012522	0.142857	0.857143	0.001957
35	3	0.005367	0.010733	0.5	0.5	0.001678
36	1	0.001789	0.005367	0.333333	0.666667	0.000839
37	0	0	0.003578	0	1	0.000559
38	0	0	0.003578	0	1	0.000559
39	2	0.003578				0

Table 4.9. Percentage of calves in the Florida manatee population, obtained through different studies, 1976-91.

Source	Method	Area	Classification	Proportion
Powell (1981)	Field identification during winter	Crystal River	Suckling and in attendance with their mothers	18.5 (1976-77) 10.2 (1977-78)
Powell and Rathbun (1984)	Year-round aerial surveys	Crystal River		$x = 10.9$ (1977-78/1981-82)
	Field identification during winter	Crystal River		$x = 11.0$ (1978-79/1981-82)
Rathbun et al. (1990)	Year-round aerial surveys	Crystal River	< 2m length, closely associated with a larger animal	Sep-Mar $x = 11$ Nov-Feb $x = 8.2$ (1981-82/1984-85)
	Field identification during winter	Homosassa and Crystal Rivers	Individual recognition	12.3 (1978-78/1983-84)
Reynolds (1992)	Winter season aerial surveys	Statewide	< 1/2 length of closely associated animal	$x = 9.6$ (1977-78/1991-92)

Table 4.10. Representation of each age class in the age-at-death sample of Florida manatees for three different periods (one-way ANOVA: $F = 1.97$, $df = 2$, $P = 0.1393$).

Age class	Period		
	1976-80	1981-85	1986-91
0	25.9	22.1	28.5
1	12.7	17.4	17.0
2	9.6	9.9	9.3
3	6.1	7.3	4.9
4	3.0	3.2	2.5
5	3.6	2.0	3.7
6	4.6	3.5	3.7
7	3.0	4.1	5.1
8	4.6	4.1	2.6
9	3.6	2.3	1.8
10	2.5	3.8	2.1
11	2.5	2.3	1.6
12	2.0	1.5	2.5
13	2.0	1.2	1.8
14	1.5	1.7	1.2
15	0.5	0.9	0.7
16	2.0	2.0	1.6
17	0.5	1.2	0.7
18	0.5	0.9	0.9
19	2.0	1.2	1.1
20	0.5	0.3	0.7
21	0.5	0.9	0.7
22	1.5	0.6	0.5
23	0	0.3	0
24	0	1.7	0.9
25	0.5	1.7	0.7
26	0.5	0.3	0.7
27	0.5	0.3	0.5
28	0.5	0.9	0.4
29	1.5	0.3	0.4
30	0	0	0.2
34	0	0	0.2
35	0.5	0	0.4
36	0	0	0.2
37	0	0.3	0.2
39	0	0	0.4
59	0.5	0	0.4

Figure 4.1. Distribution of age classes of Florida manatees affected by the various agents of mortality, 1976-91. Squares represent the mean \pm standard error, and bars mark the range, and numbers above lines represent sample size.

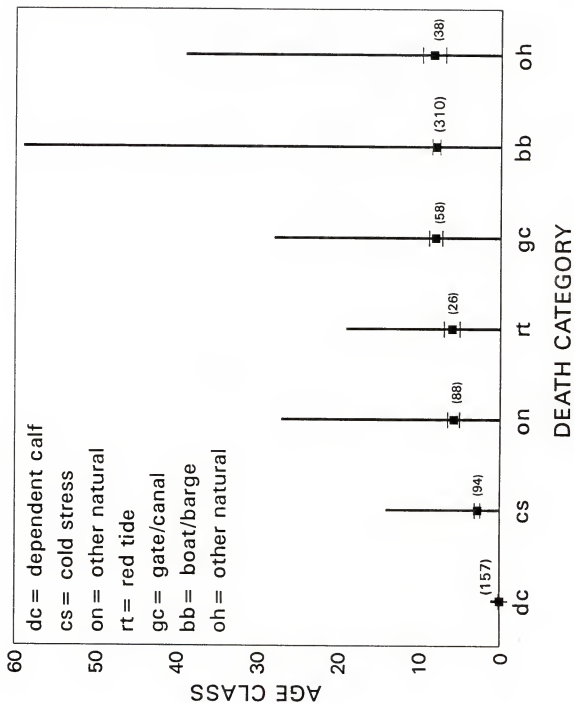


Figure 4.2a. Proportion of each age class of Florida manatees affected by natural mortality agents, 1976-91.

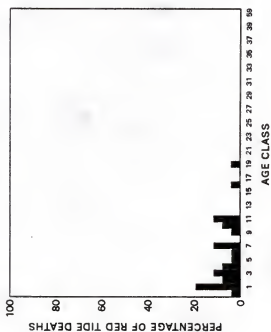
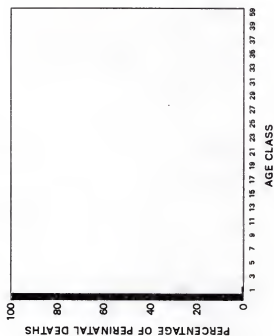
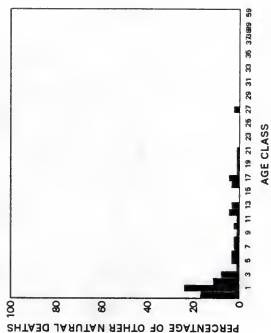
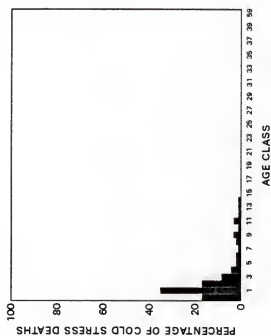


Figure 4.2b. Proportion of each age class of Florida manatees affected by human-related or undetermined causes of death, 1976-91.

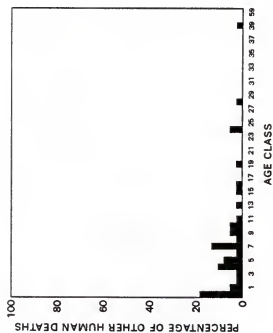
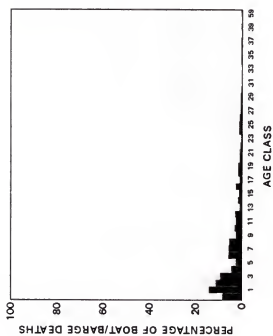
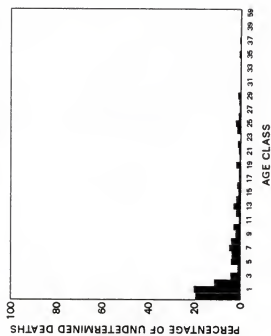
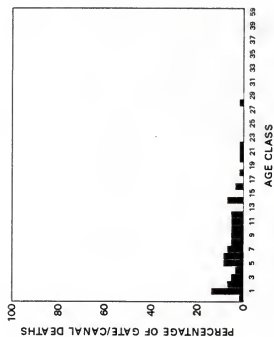


Figure 4.3. Survivorship curve for Florida manatees recovered in the southeastern United States based on a life table built for the period 1976-91.

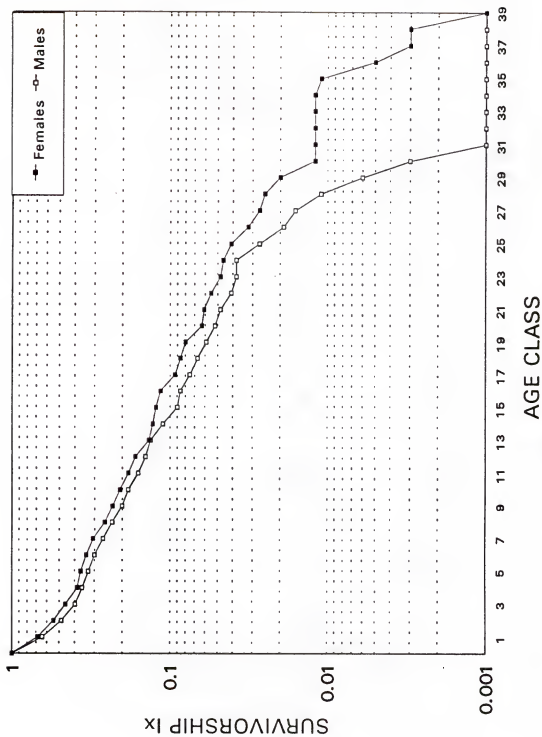


Figure 4.4. Representation of the 4 life-table schedules of male and female Florida manatees, 1976-91, based on the life table.

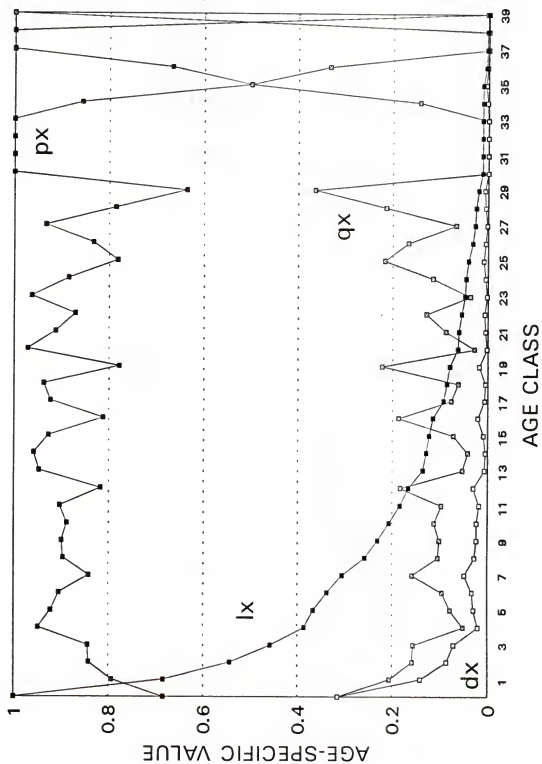


Figure 4.5. Relationship between age-specific survivorship (l_x), fecundity (m_x), and net reproductive rate ($l_m m_x$) in female Florida manatees, 1976-91.

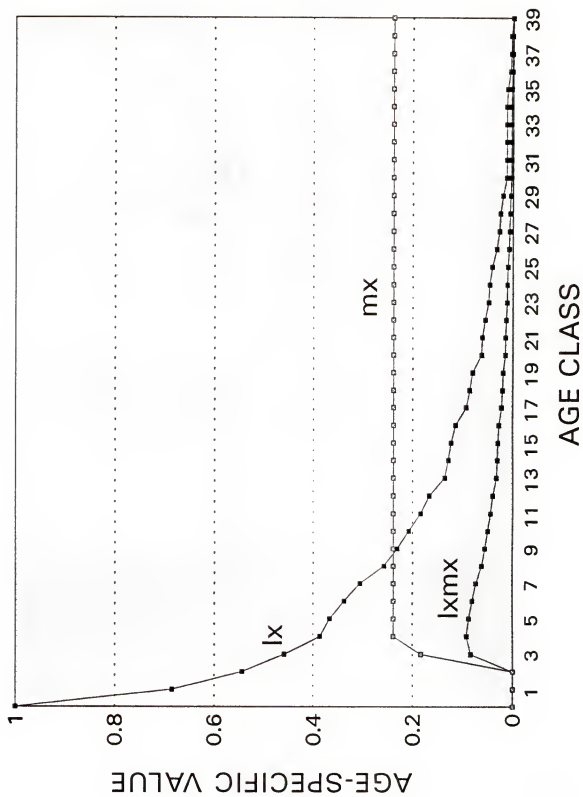
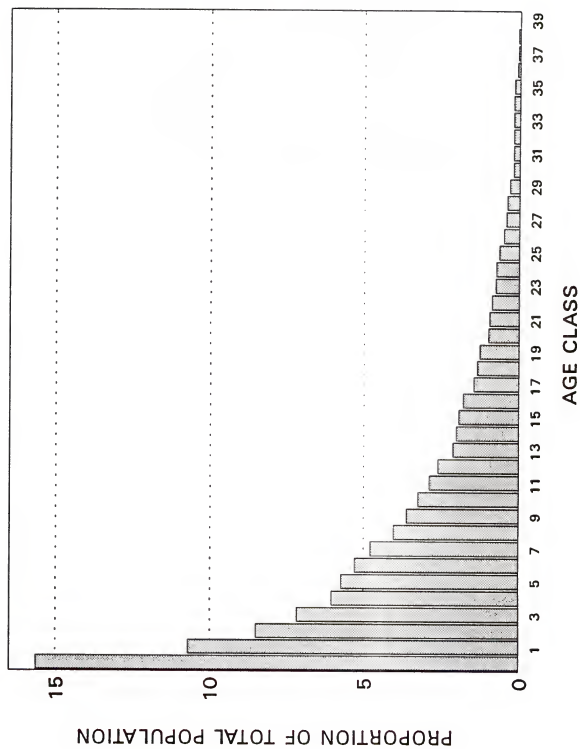


Figure 4.6. Stable age distribution calculated from a sample of ages-at-death of female Florida manatees, 1976-91.



CHAPTER FIVE
POPULATION VIABILITY ANALYSIS

Introduction

The field of demography is concerned with the dynamics and structure of populations. The high adult survival and low fecundity rates of large mammal populations inhibit direct observation of population changes over time and demand an alternative way to understand population dynamics. Development of a population model through computer simulations based on age structure, mortality, and fecundity schedules can provide insights into demography and life history and permit predictions of future trends, projections of effects of potential events and evaluation of alternative management options. Population viability analysis requires a considerable amount of data and reliable estimates of population parameters to allow meaningful interpretation.

Longevity, population age structure, mortality, and age-related aspects of reproduction are key elements of population biology that have been poorly understood but that influence the ability of a population to perpetuate itself. The estimation of such parameters constitute basic data for modeling the Florida manatee population, a task mandated in

the revised Florida Manatee Recovery Plan (U. S. Fish and Wildlife Service, 1989).

In this chapter I use field data collected over a 16-year period to examine the dynamics of the Florida manatee population given a set of parameter and variability estimates. Through a series of computer simulations, I determine the sensitivity of demography to changes in various parameters, evaluate the influence of environmental and demographic stochasticity on the statistics, and predict the population outcome after a long period of time. Unlike life tables, which provide average long-term projections of population growth, stochastic models indicate fluctuations in population size that result from variability in the demographic parameters.

Materials and Methods

Population modeling draws heavily on reproductive and mortality parameters. Demographic parameters relevant to modeling include age structure, sex ratio, and age-specific fecundity and mortality. The computer package VORTEX51, designed for population-viability analysis, combines analytic and simulation techniques. VORTEX was used to calculate the fate of manatee populations relative to persistence or extinction under various sets of population parameters. VORTEX is a Monte Carlo simulation of the effects of both deterministic and stochastic events

(demographic, environmental and genetic) on populations (Lacy, in press). Demographic stochasticity includes random variation in demographic parameters, while environmental stochasticity refers to variation in the physical environment (Simberloff, 1988), although the distinction is somewhat arbitrary (Goodman, 1987a). The first is said to only affect very small populations (Goodman, 1987a, b; Leigh, 1981), whereas the latter may be significant to larger populations (Goodman, 1987a, b; Leigh, 1981; Mode and Jacobson, 1987). VORTEX allows for the input of the proportion of reproducing individuals, and the proportion dying in each age class, as well as standard deviation in estimates of birth and death rates to allow modeling of environmental variation. With the variables allowed to change at random, the program provides risks of extinction at specified intervals (e.g., every 100 years during a 1,000-year simulation), probability of persistence through the total period, mean time to extinction of those simulated populations that went extinct during the specified period modeled, and mean final population size by the end of the same period. Standard deviation values for extinction time and final population size indicate the range of results across the runs. Standard errors of probability of survival are small, given by

$$\frac{P * (1-P)}{\sqrt{1000}}$$

(Lacy et al., 1989).

Uncertainty associated with some of the estimates was handled through bracketing of values, resulting in the evaluation of several different scenarios on the probability of extinction.

Each demographic parameter was assigned a distribution with a mean and standard deviation to allow modeling of environmental variation. The program uses binomial distributions to model annual fluctuations in probabilities of reproduction and mortality (Lacy, in press). Input values for age-specific death rates were values obtained from the female life table (Chapter 4) reduced by 11% to approximate the $r = 0$ assumed in the former method. Percent mortality for age class 0 was 28.2 (SD = 7.7); for age class 1, the value was 18.36 (SD = 10.2); for age class 2, 14.05 (SD = 7.9); and for age class 3, 13.9 (SD = 6.74). Mean adult mortality was modeled at 9.22% (SD = 1.24). Reproductive values were calculated based on carcass recovery, field examination and histological preparation, and proportion of females as noted in Chapter 3. Maximum litter size was 2. The proportion of females producing x offspring per year was recalculated as the proportion observed pregnant plus $1/2$ of the proportion observed to be lactating, to correct for those lactating females who had

given birth recently. Very little twinning has been observed in manatees (Chapter 3; Rathbun et al., in manuscript); 4% of the reproducing females were assigned to producing twins every year. Input values were 57.67% producing 0 offspring, 40.64% producing 1 offspring, and 1.69% producing 2 offspring (SD = 13.2). The population was truncated at age class 39. Manatees are polyandrous, the female mating with a number of males (Hartman, 1979). The percentage of males actively reproducing (45%) was based on male age structure and the findings of Hernandez et al. (in manuscript) on sexual maturity, where 93% of mature-sized specimens in non-winter season presented sperm and approximately 50% of males are in the mature size category. Sex ratio was assumed to be 1:1 (Chapter 4). Internal consistency was checked by comparison with results from the life table. Values for environmental variation associated with mortality and reproductive estimates were calculated based on year-to-year standard deviation of those rates and maintained constant for most schedules (unless specified).

Current (initial) population size was based on the maximum number of manatees (1,856) counted during aerial surveys in early 1992 (Ackerman, 1992). The value is believed to be a minimum figure due to visibility and absence bias (Lefebvre et al., 1992; Packard et al., 1985b). Populations of initial size of 2,000 were therefore modeled as a minimum value to evaluate the impact of changes in

different estimates on population growth and persistence. An alternative population size was also modeled: 4,000. There are no figures for the number of manatees that could live in Florida under the present conditions. The species has probably never been bountiful in historic times (O'Shea, 1988), and the extension of seagrass beds and proliferation of exotic aquatic plants, and lack of signs of food stress suggest that manatees are below carrying-capacity levels. Carrying capacity was arbitrarily modeled at levels of 5,000 and 8,000 and 10,000. Carrying capacity was also modeled with environmental variability (arbitrarily set as 10% of the initial population or 200 individuals), to introduce variations in weather conditions and longer-term habitat changes (Eberhardt, 1971). When simulating two subpopulations, I used the above reproductive parameters, and survival rates obtained from the life table (Chapter 4). Initial population size was 1,000 and carrying capacity was 2,500 for each coast.

Variability due to periodic cold stress and due to occasional diseases and parasites was built into the environmental variation estimate. Catastrophes not included in this variability were assumed to occur more rarely and with greater impacts on the population. Three catastrophic events were modeled: (1) episodes of extreme cold fronts, (2) high-intensity hurricanes, and (3) disease outbreaks. Data indicate that a major winter freeze (reaching average

temperatures of -6°C) occurs every 60 to 90 years (Chen and Gerber, 1990). Using an average of 75 years, the occurrence of an extreme cold event was modeled with an annual probability of 1.33%. The severe cold front in late December 1989 killed at least 55 manatees in December 1989-January 1990 (Florida Department of Natural Resources, unpublished data). Assuming a total population of 2,000, that figure corresponds to 3% mortality; considering that an extreme freeze could kill three times as many manatees (9%), a 10% level was modeled for effects on mortality. Effects on reproduction are unknown, but are likely to be less damaging than on survival. Even when breeding is reduced for a season, long-lived species are capable of reproducing in the following years (Seal and Lacy, 1990). For females giving birth in the spring, some proportion of the pregnancies may result in miscarriages, so an arbitrary level of 5% was modeled.

Hurricanes affecting Florida tend to concentrate mostly in the Panhandle and in southern Florida. Of 11 major hurricanes in this century only one was class 5 (winds of $248+ \text{ km/h}$, storm surge of $5.5+ \text{ m}$) (Johnson and Barbour, 1990). Analyzing frequency curves for different intervals (based only on the west coast of Florida), Ho and Tracey (1975) calculated a recurrence of 200 years for storms of such magnitude. No data are available on hurricanes and manatee mortality, but dugongs have been stranded by cyclone

tidal surges (Heinsohn and Marsh, 1977; Marsh, 1989). In the future, a combination of increased sea-level rise with a class-5 hurricane could have devastating effects (Hine et al., 1987). Destruction of seagrass beds may cause indirect mortality in dugongs (Heinsohn and Spain, 1974). Hurricane Camille destroyed 33% of the seagrass beds in Mississippi waters (Eleuterius and Miller, 1976). Wave action may also produce upwelling, causing water 5°C colder than normal to remain for days (Simpson and Riehl, 1981). The interrelation of all these factors is hard to estimate without background data, but I assumed 10% of the population would be directly affected by the storm, and 5% would suffer deleterious effects in reproduction.

The impact of disease was modeled as a catastrophe capable of decimating all the animals in a warm water aggregation, a number varying between 200 and 300 (Reynolds and Wilcox, 1986). A 90% index was given for survival (10% reduction), and 95% for reproduction, with a probability of 2%. The probability of a disease agent spreading to other warm-water concentrations was modeled as 1% with a 70% impact on survival and 85% on reproduction, and 0.75% with a 50% impact on survival and 75% on reproduction. These probabilities were estimated as projection on a 1,000-year period; in reality, in shorter term, these probabilities are much higher.

Even though large mammals have density-dependent regulatory functions (reproductive rate of adult females, age of first reproduction, immature mortality rates, and adult mortality rates) (Charnov, 1986; Eberhardt, 1977; Fowler, 1981a, 1987; Fowler et al., 1980) no density-dependence was chosen because: (a) populations with numbers reduced due to human impact are far from a natural limitation on their size (K), so it is reasonable to model them without density dependence (Ferson and Akçakaya, 1990), and (b) generally, models without density dependence are conservative estimators of extinction risks, giving higher estimates of risks than the more realistic models with density dependence (Ferson et al., 1989; Ginzburg et al., 1990), even though this is not always true (Boyce, 1992).

A summary of the default parameters used in the simulations is presented in Table 5.1. For each set of parameters, I set the program to run for 1,000 years and replicated the simulation 100 times. The scenario with the estimates believed closest to manatees' current demography and minimum population size was considered the basic scenario. Subsequently I altered values of the various estimates one at a time by 10%, and repeated the process (Table 5.2). Table 5.3 shows simulation results for the evaluation of a manatee metapopulation, modeling 2 subpopulations (Atlantic and Gulf coast) and low levels of migration (0.01%/year).

I also simulated the basic scenario for 100 years, 100 times, to evaluate the status of the manatee population under Mace and Lande's (1991) system. That system uses as criteria broad categories of population size, immigration rate between population fragments, percentage decline in a number of generations, and probability of catastrophes.

Results

Deterministic and Stochastic Scenarios

The simulated schedule with calculated average mortality and reproductive rates and without environmental variation or catastrophes (scenario 1, Table 5.2) yielded a growth rate of 0 (Fig. 5.1). The probability of persistence through 1,000 years was 1.0, and the final population was approximately 1.5 times as large as the initial. In a more realistic schedule, believed to approximate the present situation, the introduction of year-to-year environmental variation on the demographic parameters and predicted frequency and severity of extreme cold fronts, high-intensity hurricanes, and disease (scenario 4, Table 5.2) resulted in a slightly negative growth rate, but a much lower probability of persistence in 1,000 years was predicted ($P = 0.44$) and the mean final population size projected was only 10% of the original value (Fig. 5.2). The increasing magnitude of disease had the effect of decreasing the projected persistence time of the population.

For example, scenarios 13 and 14 were planned to simulate a disease spreading to more than one aggregation (occurring with a lower probability but with higher impact on the population).

Sensitivity to Variation in Demographic Parameters

The most important factors for the prospects of the population were mortality and fecundity, and the model was most sensitive to changes in adult survival. The mean time to extinction for populations simulated to go extinct within 1,000 years under most schedules was less than 600 years, with probabilities of persistence ranging from 9 to 44% (Table 5.2). The persistence probability became null and the ultimate population size was reduced to 0 when a larger proportion of adult manatees were simulated to suffer mortality (scenario 10, Table 5.2). Adult mortality had a 2-times greater impact on r than any of the juvenile age classes except age class 0 (scenarios 6-9, 10; Table 5.2) resulting in total extinction (mean final population size = 0; Fig. 5.3).

In contrast, if adult manatee mortality could be reduced by 10%, population growth rate would improve from negative to positive 3% as compared to the basic scenario (scenario 12; Fig. 5.4). If all age classes suffer an increase 10% in mortality level as compared to present levels the population net growth would decline to -1.8% and

final population size would again be reduced to zero (scenario 11, Table 5.2; Fig 5.3).

If adverse effects in the population decreased reproduction output by 10%, population growth would become $r = -0.012$, and 1,000-year persistence probability would be null again (Fig. 5.5). If age-specific fecundity decreased after a certain age the probability of persistence would similarly be considerably reduced.

Simulation results indicate that a population of initial size 2,000 has 44% chance of persistence in 1,000 years (scenario 4, Table 5.2) but if the initial size is twice as large probability of persistence increases by 50% (scenario 18, Table 5.2).

Carrying capacity (K) did not affect the growth rate, but the probability of persistence, and the size of the persisting population increased with increasing K (scenarios 4, 16, and 17; Table 5.2).

If the population on both coasts of Florida were completely isolated (and assuming equal probability of catastrophes), probability of persistence would decrease ($P = 0.22$, scenario 1, Table 5.3). However, persistence probability reflects the default value for the total population (scenario 2, Table 5.2) when a small amount of migration (0.01%/year) is allowed between the east and west coasts. It is likely that both coasts differ between probabilities of being affected by catastrophes. If the

probability of cold events and disease spread were higher than the default value for total population on the east coast, and the probability of a hurricane strike is larger on the west coast, then population growth rate would decrease to -0.004% , and persistence probability fall to 25% , despite migration.

Discussion and Conclusions

Population viability is the outcome of the interaction of population parameters in a particular environment, where persistence requires that reproduction outbalances mortality. Population viability analysis, an expansion of the original concept of minimum viable populations (Shaffer, 1981) is a process by which the persistence of a population can be predicted with a certain probability level over a given time-interval (Gilpin and Soulé, 1986; Soulé, 1986, 1987a). Analyses of viability have been applied to a number of endangered and threatened species or small populations of vertebrates, including marsupials, carnivores, ungulates, primates, birds, some species of reptiles and amphibians, and plants (Kinnaird and O'Brien, 1991; Lacy, in press; Lande, 1988; McKelvey et al., 1992; Menges, 1986; Murphy et al., 1990; Shaffer, 1983; Shaffer and Samson, 1985; Taylor, 1992), but never to any marine mammal. Viability analysis does not project exact extinction or persistence times. Its importance lies in the identification of those factors that

contribute most to the population growth or decline. It allows safe and expeditious examination of conservation strategies by desk-top variation of individual population elements, and evaluation of the population outcome in terms of probability of persistence. The range of manipulations allowed by the simulations would be prohibitive in the wild, given manatees' longevity and level of endangerment.

The manatee simulations suffer from some of the same problems identified for the grizzly bear model based on a 12-year data base (Shaffer, 1983): (a) the period of data collection is not extensive relative to longevity, so long-term cyclical patterns cannot be detected; (b) despite being the best available data set, it is not error-free, and one cannot separate sampling variation from true variation in the basic parameters; (c) the model does not include genetics; and (d) the model does not include density dependence.

Vulnerability Factors

An understanding of the various simultaneous forces acting towards extinction is necessary to attempt conservation of endangered species (Shaffer, 1981, 1990; Soulé, 1987b; Mace and Lande, 1991). Large mammals are characterized by high adult survival rate, low fecundity with high parental investment, long generation time, long life spans, usually late maturity, and population size

rarely exceeding carrying capacity (Fleming, 1979; Goodman, 1981; Laws, 1981; Stearns, 1976). A measure of the importance of a given vital rate and the effects of environmental perturbations may be obtained by analysis of the population's responses to variations in the parameters (Caswell, 1989). Manatee dynamics show greater variability in reproductive rates and survivorship of young age classes, and greater sensitivity of population growth rates to changes in adult mortality and fecundity. In the typical long-lived mammal, reproduction and the survivorship of young are the components of the life table most sensitive to environmental change, whereas adult survival is the least variable and consequently more difficult to detect (Caughley, 1977). However, adult mortality has the greatest effect on the annual rate of increase (Eberhardt, 1977; Fowler and Smith, 1973; Marsh et al. 1984b; Packard, 1985b). Adult survival may be critical to population maintenance in other long-lived taxa as well (Croxall et al., 1990; Grier, 1980, 1982; Harding, 1991).

Persistence Time and Population Projection

Time to extinction provides a measure of the population's susceptibility to demographic challenges (Lacy et al., 1989). Usually, for endangered species, a risk of extinction of $\leq 5\%$ in 100 years is taken as a goal for preservation of the species from extinction (Seal and Lacy,

1990). Distinction must be made between resilience (short-term viability in face of normal events), fitness (short- and medium-term survivability) and adaptability (long-term survival) (Salwasser et al., 1984). To achieve effective recovery and guarantee that evolutionary and adaptive processes can progress, persistence over 1,000 years is an appropriate time horizon for a long-lived species such as the manatee. Shaffer (1981) adopted a 99% persistence probability for 1,000 years as a viability criterion for grizzly bears. Population persistence times are determined by the population size, growth rate, and the population's spatial distribution (Lacy et al., 1989). In turn, growth rate is determined by life history characters and demographic, environmental and genetic stochasticity (Shaffer, 1981).

Variability in survivorship and fecundity estimates is often associated with environmental variation through time (Bessinger, 1986; Caughley, 1966; Murphy and Whitten, 1976). Normal, random changes may reduce but do not often eliminate populations unless numbers are already depleted by deterministic factors (Gilpin and Soulé, 1986). Long-term data on age-specific reproduction in manatees did not demonstrate significant changes over time (1976-91; Chapter 4). Variability in survival estimates was similarly small for each age class, especially in the adult segment, as in other large mammals (Fowler, 1981a; Laws, 1981). However,

the incorporation of catastrophes into Florida manatee demographic parameters resulted in a decreasing population (scenario 4 in Table 5.2, $r = -0.003$). As extremes of environmental variation (Simberloff, 1988), catastrophes have been implicated in the extinction or near extinction of populations (whooping crane: Doughty, 1989; reindeer: Klein, 1968; black-footed ferret: Seal et al., 1989; Puerto Rico parrot: Lacy et al., 1989). The prospect of a disease spreading among manatees indicates that the manatee population could reach a point where the total population is so small (and migration between the two coasts so negligible) that random variations in demographic parameters may come into effect, genetic variability would likely be reduced, and animals might encounter difficulty in finding mates (Allee effect). The combination of such parameters would lead a population to extinction.

Only under constant conditions (no environmental variation), inexistence of catastrophes, or very large population sizes does the manatee population have high chances of surviving in the long term, assuming $r = 0$ (scenarios 1-3; Table 5.2). For populations experiencing extinctions, mean final population level is low, and projected mean persistence time under present conditions is only moderate, partially due to high longevity, and because populations of large iteroparous organisms are physiologically protected against short-term environmental

change (Gilpin and Soulé, 1986). However, persistence time must be viewed with caution, due to its probabilistic nature and approximately exponential distribution; these factors imply that the population might go extinct prior to the calculated mean persistence time (Lacy et al., 1989; Seal and Lacy, 1990; Stacey and Taper, 1992). Approximately 2/3 of the simulations will results in extinction time of final population size within ± 1 SD (standard deviation) of the mean.

Population size imposes an obvious effect on the vulnerability of the Florida manatee population. Larger populations have higher probability of persistence and are less affected by environmental variation. If the manatee population level is close to the count from the synoptic survey (1,856, Ackerman, 1992) used in the basic scenario ($N = 2,000$, scenario 4, Table 5.2), extinction might occur at approximately 750 years.

General guidelines have been set for minimum viable population sizes (Franklin, 1980; Gilpin and Soulé, 1986; Lande, 1988; Simberloff, 1988; Soulé, 1987a; Soulé and Simberloff, 1986). Soulé (1987b) suggested a number in the low thousands, but given stochasticity, large mammals may require several thousands to attain viability (Lacy et al., 1989; Thomas, 1990). A desirable population level should be one, given the natural fluctuations, unlikely to fall into an extinction vortex. For example, a mean final manatee

population of less than 100 (scenarios 6, 7, 8, 14, Table 5.2) is demographically unstable, and therefore vulnerable to random demographic processes. The International Union for the Conservation of Nature (1988) recommends the implementation of captive breeding programs of populations that fall below the threshold of 1,000 individuals. The term mean further implies that the population will have fallen below that level for a number of instances.

Most of these guidelines have been based on theory and simulation models, because the direct observation of natural population dynamics of large mammals is hampered by their longevity. Berger (1990) confirmed the prediction of greater vulnerability of smaller populations by examining historical data on over a hundred populations of bighorn sheep (*Ovis canadensis*).

A proposed reevaluation of IUCN criteria to list species, based on general principles of population biology and believed appropriate for most vertebrates, examines levels of probability of extinction (critical, endangered and vulnerable) in a 100-year period (Mace and Lande, 1991). The authors judged that 100 years is more realistic in terms of prediction accuracy and legal expectations. Manatees did not meet the minimum criterium of 10% probability of extinction in 100 years for the lowest level (vulnerable) defined by Mace and Lande (1991), but 100 years is inadequate to evaluate extinction probability for a species

whose longevity is over half of that period. The proposed system should be used as an exploratory tool, especially in the case where scarcity of information precludes the use of more detailed models.

The magnitude of environmental variation is important in determining persistence times and indicates that manatees are less likely to go extinct under stable than unstable environmental conditions (scenario 15).

Occasionally catastrophic events may occur that influence one age class in particular or the population as a whole. Cold episodes affect mainly young animals, but a more prolonged cold spell, associated with a potential shutdown of power plants, might affect both a larger percentage and a wider range of age classes. Manatees have learned to rely on sources of warm water during the winter. There are indications of the influence of the interruption of power plant operation on manatee distribution and movement (Packard et al., 1985a). Temporary malfunction (shutdown), intermittent or termination of operation may jeopardize manatee survivorship in the area (Campbell and Irvine, 1981), and managers must be notified of any alteration in the operational schedule of power plants during the winter (Packard et al., 1985a). For plants approaching the end of their life span, a program must be devised to allow manatees to learn alternate sources, or guarantee the discharge of warm water. The contingency plan

must include recommendation/actions to be taken in case of a shutdown concurrent with severe winter.

The large aggregations of manatees around warm-water sources during the winter sets the stage for catastrophes to occur (O'Shea and Ludlow, 1992). An oil or chemical spill, or the introduction of a parasitic or epizootic agent, or a toxin in the food base into the population at one of these concentrations could produce calamitous mortality, and so could indirect destruction of the localized winter food supply. Oil contamination may affect manatees directly or through their food species (Brownell et al., 1978; St. Aubin and Lounsbury, 1990). Susceptibility to infectious disease could be enhanced by debilitation of the immune system due to contaminants (Hall et al., 1992a; Heuschele, 1991; Geraci and Ridgway, 1991) and possibly thermal stress. Disease is the most unpredictable factor. Contrary to historical records of incidence of hurricanes and extreme cold winters, there are no records for the occurrence of disease epizootics in manatees, so they are modeled with most uncertainty. However, the likelihood of their occurrence and potential impact must not be underestimated. If their effects are not devastating, then the population has a reasonable level of security; however, if catastrophes have a strong effect (scenario with 50% mortality) or occur with higher frequency than modeled, then the population would face a much greater risk of extinction. Harwood and Hall

(1990) roughly estimated the impact of disease on marine mammals to elevate average mortality by 15-30% of the regular levels. Simulations conducted here show that such levels would have a significant negative impact on the rate of increase of the population.

The red tide episode (O'Shea et al., 1991) shows that one factor, acting in a restricted area, may affect a considerable proportion of manatees in a particular region. If the record count of 338 manatees at the Fort Myers power plant in winter 1984-85 (Reynolds and Wilcox, 1986) is an indication of manatee abundance in southwestern Florida, the red tide incidence affected more than 10% ($n = 37$) of the animals using that area. A natural disaster may affect populations over a considerable period of time as well. Indirect and direct mortality as a consequence of cyclones have been documented for dugongs (Heinsohn and Spain, 1974; Marsh, 1989), and longer-term effects have been suggested (Jones, 1967).

The spatial distribution and possibility of recolonization are often taken into account when assessing wildlife population viability (Lacy et al., 1989). Manatees are very mobile (Reid and O'Shea, 1989) and live in an environment with few barriers to long-distance movements. Therefore it is hard to envision a metapopulation structure (Harwood and Hall, 1990), except perhaps for the east and west coasts of Florida. If a disaster such as a cold front

or hurricane were to decimate a large proportion of the population on either coast, replacement primarily would be limited by the low potential growth rate. Replacement through migration would be slow as well: manatees are not common in the Florida Bay or Florida Keys (Hartman, 1974) and are believed not to travel regularly around the southern portion of the peninsula. Although some individuals probably move across the state via Lake Okeechobee, such movement has never been recorded (O'Shea, 1988). Furthermore, the Florida manatee population is not likely to receive migrants from the Antillean subspecies. Physical factors such as strong currents in the Florida Straits and cooler temperatures in the northern Gulf of Mexico are believed to have promoted genetic isolation between the two subspecies of West Indian manatees (Domning and Hayek, 1986).

Dynamics may vary for the same species from place to place, reflecting the differences in environmental conditions and external factors; however, no differences were detected among mortality factors between the Atlantic and Gulf coasts.

Management Implications

The goal of the recovery plan prepared for manatees (U. S. Fish and Wildlife Service, 1989) is to downlist the species from endangered to threatened. In other words, the

risk of extinction must be reduced to an acceptable level, compatible with spatial persistence and ecological fitness. Results from this study indicate that manatees are still at high risk of extinction in the long term. Even under the Mace and Lande proposal, it would be premature to modify the status without longer monitoring (Mace and Lande, 1991).

The size of Florida's human population is expected to increase >2% a year through at least the year 2,000 (Bureau of Economics and Business Research, 1991). Most of the population settles on the coast, and a large number will practice water sports. Boat registration numbers have increased by almost 4%/year (Ackerman et al., 1992). More boats in the water represent an increased possibility of accidents. As boat mortality does not seem to improve with age, and over 50% of the manatee population consists of adults, adult manatee mortality is likely to increase. This could only worsen the present prospects, as increased adult mortality leads to the shortest time to extinction (scenario 10, Table 5.2).

Because random processes are beyond the control of wildlife managers, the problem must be approached from the angle of increasing the Florida manatee population growth rate. To improve the assumed situation of zero growth and to ensure the maintenance of the population over time in the face of variability and environmental stresses, reproductive effort would have to be increased and/or mortality must be

decreased. The percentage of females reproducing may have increased over the past 15 years, and the rate of offspring production is probably at the maximum potential (Chapter 3; Rathbun et al., in manuscript; Reid et al., 1992). Areas important for calving must be identified and protected as critical habitat to allow realization of the full reproductive potential. It is essential that the protection of feeding habitats be implemented as well. The importance of nutritional conditions to reproduction has been demonstrated for a number of large mammals (Knight and Eberhardt, 1985; Sadleir, 1969; Widdowson, 1981). However, reducing mortality, particularly of adults, is probably the most effective course of action available. Clearly, it would be almost impossible to insure completely against cold-stress mortality, although ensuring that power plants remain operational or discharging warm water would prevent jeopardy to animals seeking such refuges during cold spells. Even if they were preventable, most of the cold-related deaths occur in the age classes 0 through 2 (Chapter 4), where mortality variation has a lesser impact on persistence than in adult age classes.

The most feasible solution is to reduce human-related mortality. If boat-related mortality (the most important mortality factor) were removed or eased, more females would live into older age classes (where survivorship is naturally elevated), contribute more in terms of reproductive output,

and consequently improve the population's rate of natural increase. If that were attained, the probability of persistence of the population would increase considerably.

If the stationarity modeled in this study represents the situation of the wild manatee population, it is clear that the population could not support any added mortality, and stochasticity is certain to play a role in decreasing chances of long-term persistence. Present survival levels will not protect manatees against catastrophic loss such as hurricanes, freezes, and disease. Disease epizootics have caused high mortality in wildlife populations (Burrows, 1992; Davis et al., 1981; Hall et al., 1992b; Thompson and Miller, 1992; Thorne and Williams, 1988). Viral epidemics, parasites and toxins have caused mortality levels of up to 97% in some populations of marine mammals (Harwood and Hall, 1990). The increased interactions between cetaceans and humans have raised concern as to disease transmission (Geraci and Ridgway, 1991). Although no epizootic agent has been identified so far in manatees (Forrester, 1992), as human population in Florida grows and more people are attracted to swim and interact with manatees, the opportunities of disease transfer between manatees and humans or domestic animals can only increase. *Toxoplasma gondii*, a protozoan parasite that may infect birds and mammals, including man (Hammond and Long, 1973) has been implicated in the death of a manatee (Buergelt and Bonde,

1983). Although some authors may not grant great importance to wildlife disease (Scott, 1988), and disease may act as a natural selection agent (Jones, 1982), it is undeniable that potential effects of disease must not be overlooked when dealing with an endangered species. It is recommended that efforts be intensified to determine the kinds of diseases manatees are exposed to. Such knowledge should help managers better respond in the event of disease outbreaks.

Present management measures include regulation of speed zones, sanctuaries, rehabilitation of injured animals, and public education. It is essential to assure high adult survivorship (above 90%) to maintain the population of Florida manatees. The addition of a few percentage points in adult mortality can drastically change the population dynamics of manatees and result in an unstable or decreasing population. Based on the simulations performed and factors affecting the manatee population, ideally one needs a very large manatee population to guarantee persistence for significant periods of time.

Research Needs

Population viability analyses are inherently speculative and predictions are only probabilistic (Shaffer, 1990). Because all the factors are neither incorporated into the model (i.e., genetics, density dependence) nor totally understood, and because feedback processes are not

examined, the viability analysis probably underestimates the true probabilities of extinction. Because the interaction of the various forces may be more than additive (Gilpin and Soulé, 1986), several authors have called for the inclusion of all parameters in a comprehensive model (Boyce, 1992; Ewens et al., 1987; Samson et al., 1985; Soulé, 1987a), although Boyce (1992) also made the point that one does the best with what one has. It is essential to obtain accurate estimates of means and variances of population parameters, which underscores the need for long-term studies and extensive field research. It is advisable to continue monitoring the status, rates, and variability of Florida manatee population parameters and to conduct further viability analyses. Specifically, data on population parameters obtained from live studies of manatees in natural populations should be incorporated into viability models.

Table 5.1. Default parameters used in the simulations of Florida manatee life history.

Each population was simulated for 1,000 years, over 100 runs. No inbreeding depression was included. Reproduction was assumed to be density-independent. Environmental variation in mortality was correlated among age classes, but not with environmental variation in reproduction.

First age of reproduction: 4

Age of senescence (death): 39

Sex ratio at birth (proportion of males): 0.5

Polygynous mating: 45% of adult males in the breeding pool

Reproduction: 57.67% (EV = 13.20 SD) produce litters of size 0

40.64% produce litters of size 1

1.69% produce litters of size 2

Mortality: 28.02% (EV = 7.70 SD) mortality between ages 0 and 1

18.36% (EV = 10.20 SD) mortality between ages 1 and 2

14.05% (EV = 7.98 SD) mortality between ages 2 and 3

13.90% (EV = 6.74 SD) mortality between ages 3 and 4

9.22% (EV = 1.24 SD) adult mortality ($4 \leq \text{ages} \leq 60$)

Frequency of type 1 catastrophe (cold): 1.33%

with 95% multiplicative effect on reproduction

with 90% multiplicative effect on mortality

Frequency of type 2 catastrophe (hurricane): 0.5%

with 95% multiplicative effect on reproduction

with 90% multiplicative effect on mortality

Frequency of type 3 catastrophe (disease): 2%

with 95% multiplicative effect on reproduction

with 90% multiplicative effect on mortality

Initial size of population: 2,000

Carrying capacity: 5,000 (EV = 0.00 SD)

Table 5.2. Results from 100 simulations of Florida manatees for 1,000 years, under various scenarios of mortality, reproduction, sources of variation and risk. Basic scenario represents the set of best estimates as to demography of the species. r = rate of increase; P = probability of persistence; EV = environmental variation; AC = age class; ASM = age at sexual maturation; N = initial population size; K = carrying capacity. See text for further details.

Scenario	r	P	Probability of persistence				Mean time to first extinction	Mean final population size
			200 y	400 y	600 y	800 y	1000 y	
1. No EV, no catastrophes	0.000	1.00	1.0	1.0	1.0	1.0	1.0	2,994 \pm 1,289
2. No catastrophes, with EV	0.000	0.94	1.0	0.99	0.99	0.95	0.94	1,250 \pm 1,283
3. No EV, with catastrophes	-0.003	0.61	1.0	1.0	0.98	0.85	0.61	85 \pm 67
4. Basic (Table 5.1)	-0.003	0.44	1.0	1.0	0.87	0.72	0.44	161 \pm 184
5. -10% reproduction	-0.012	0.00	1.0	0.48	0.03	0.00	0.00	-----
6. +10% AC 0 mortality	-0.007	0.09	1.0	0.93	0.48	0.20	0.09	31 \pm 26
7. +10% AC 1 mortality	-0.005	0.15	1.0	0.96	0.74	0.37	0.15	38 \pm 30
8. +10% AC 2 mortality	-0.005	0.19	1.0	0.96	0.79	0.45	0.19	52 \pm 64
9. +10% AC 3 mortality	-0.005	0.24	1.0	0.95	0.74	0.41	0.24	108 \pm 205
10. +10% adult mortality	-0.010	0.00	1.0	0.67	0.10	0.00	0.00	-----
11. +10% mortality overall	-0.018	0.00	0.95	0.05	0.01	0.00	0.00	-----
12. -10% adult mortality	0.003	1.00	1.0	1.0	1.0	1.0	1.0	3,073 \pm 1,231
13. Disease: 1.0/0.85/0.70	-0.005	0.21	1.0	0.93	0.67	0.42	0.21	141 \pm 362
14. Disease: 0.75/0.75/0.50	-0.005	0.12	0.97	0.86	0.48	0.24	0.12	67 \pm 64

Table 5.2.--continued

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Scenario	<i>r</i>	<i>P</i>	Probability of persistence				Mean time to first extinction	Mean final population size
			200 y	400 y	600 y	800 y	1000 y	
15. <i>N</i> = 2,000; <i>K</i> = 5,000; 2EV in reproduction, mortality	-0.003	0.15	1.0	0.76	0.47	0.26	0.15	113 ± 143
16. <i>N</i> = 2,000; <i>K</i> = 3,500	-0.003	0.38	1.0	1.0	0.84	0.60	0.38	138 ± 191
17. <i>N</i> = 2,000; <i>K</i> = 8,000	-0.003	0.49	1.0	1.0	0.92	0.69	0.49	106 ± 200
18. <i>N</i> = 4,000; <i>K</i> = 10,000	-0.003	0.64	1.0	1.0	0.96	0.86	0.64	193 ± 241
19. 10% EV in <i>K</i> (=200)	-0.003	0.44	1.0	0.99	0.85	0.67	0.44	157 ± 297
20. + 10% males reproducing	-0.003	0.34	1.0	0.97	0.77	0.51	0.34	188 ± 233

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Table 5.3. Results from 100 simulations for 1,000 years each, of Florida manatee populations under different levels of migration and catastrophes. See text for further details. r = rate of increase; P = probability of persistence; population 1 = Atlantic coast, population 2 = Gulf coast; MTFE = mean time to first extinction; MFPS = mean final population size for successful cases.

Scenario	r_1	P_1	r_2	P_2	$P_{1,2}$	MTFE	MFPS
1. Single population N = 1,000, K = 2,500 Default survival, catastrophes	-0.003				0.22	695 ± 166	81 ± 103
2. Two subpopulations Migration = 0.0001 N ₁ = N ₂ = 1,000 K ₁ = K ₂ = 2,500 Default survival, catastrophes	-0.003	0.26	-0.003	0.30	0.44	754 ± 153	88 ± 101
3. Two subpopulations Migration, survival, N and K as above Effects of cold and disease 50% higher in population 1, hurricanes 50% higher in population 2 (than default)	-0.004	0.15	-0.005	0.13	0.25	740 ± 155	76 ± 77

Figure 5.1. Simulation results on the outcome of a Florida manatee population under the deterministic (life table) scenario, after 1,000 years and 100 runs. Initial population size = 2,000.

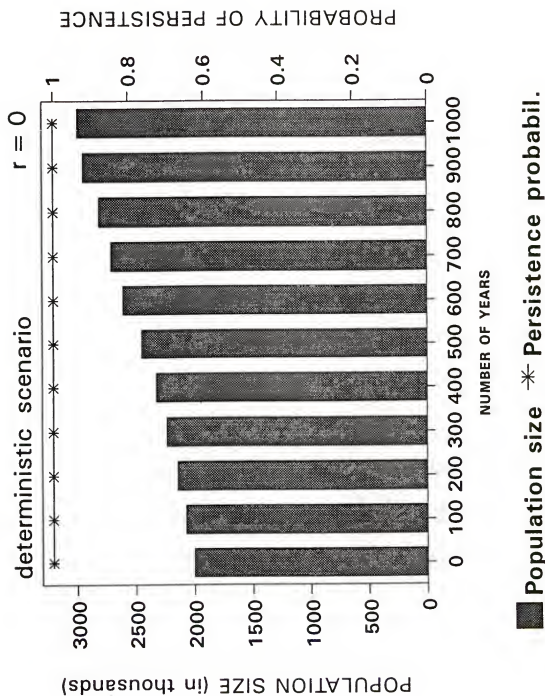


Figure 5.2. Simulation results on the outcome of a Florida manatee population under the basic (Table 5.1) scenario, after 1,000 years and 100 runs. Initial population size = 2000.

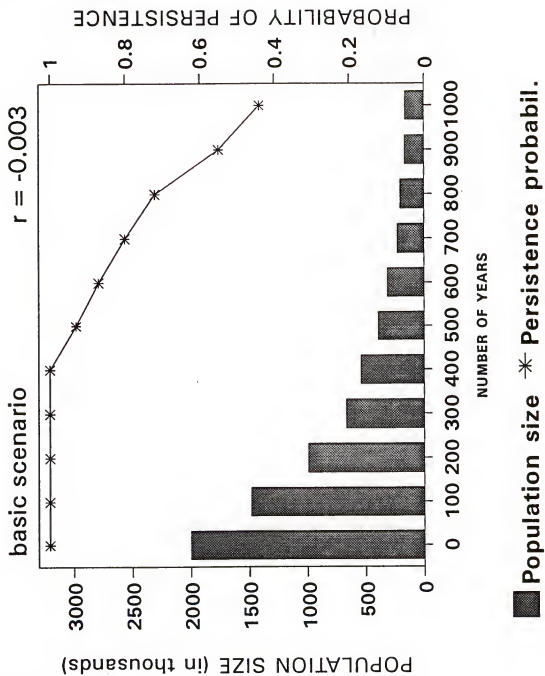
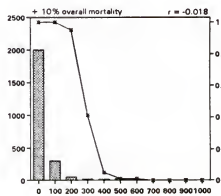
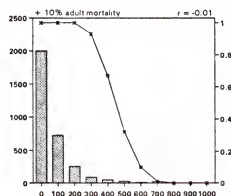
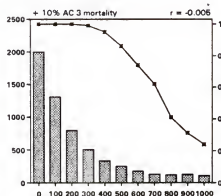
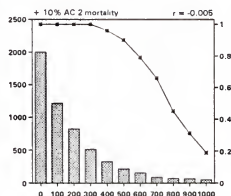
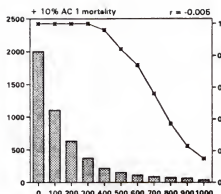
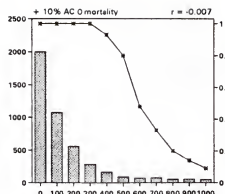


Figure 5.3. Comparison between the impact of a 10%-increase in mortality rate in juvenile and adult age classes, and over the total range of ages. Initial population size = 2,000.

POPULATION SIZE (in thousands)



PERSISTENCE PROBABILITY

Figure 5.4. Florida manatee population outcome under a scenario with adult mortality reduced by 10%. Initial population size = 2,000.

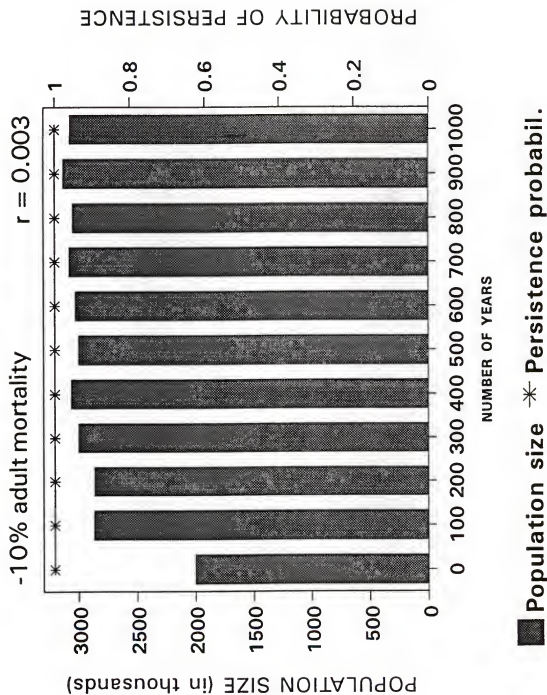
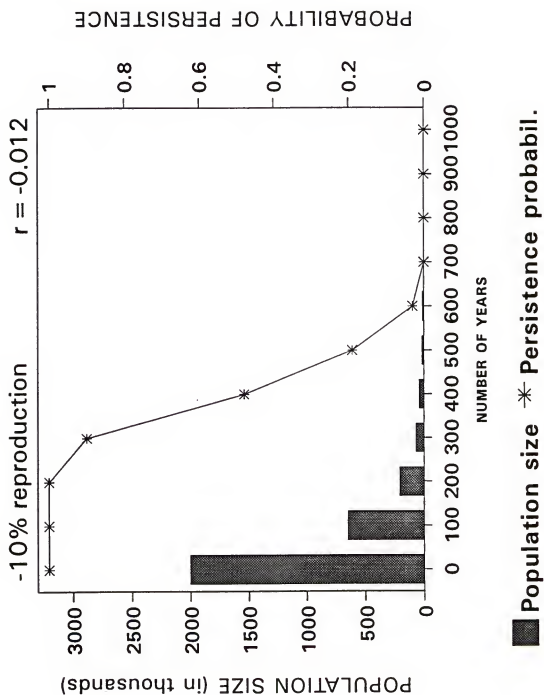


Figure 5.5. Florida manatee population outcome under a scenario of reproduction reduced by 10% relative to the basic schedule. Initial population size = 2,000.



CHAPTER SIX

CONCLUSION

Introduction

Most terrestrial and aquatic habitats are undergoing some form of alteration to accommodate increased use due to human population growth. All wildlife populations suffer some finite risk of extinction, and in geological time all species will disappear. However, current high levels of extinction are a matter of concern (Ehrlich and Ehrlich, 1981; Mace and Lande, 1991; Soulé, 1987a) given the great deviation from "background" levels (Raup and Sepkowski, 1982). Studies of dynamics of large mammals other than man are mostly restricted to game animals or species with economic or touristic value (Smith and Fowler, 1981). Very few studies of large mammal populations provide long-term detailed information on population structure and factors producing the demographic parameters. Because of the time span required to accumulate sufficient data from field studies (Smith and Fowler, 1981) at least some aspects must be based on analyses of dead animals (Laws, 1981). Data on population dynamics are needed to assess the status of animal species in general. In particular, one would like to

elucidate whether the manatee population is increasing, stable, or decreasing, and towards that effort I have analyzed data that accumulated during 16 years of a statewide manatee carcass recovery program including field necropsies and tissue sampling.

The parameters most useful in the analysis of population dynamics are those specific to age, but up until recently a method for estimating age in manatees was not available. Because of manatees' forward molar progression the routinely-used technique of counting growth-layer groups in teeth could not be used. A search for another hard structure with layer deposition indicative of age led to the dome portion of the tympano-periotic complex, which proved to contain growth-layer groups in numbers comparable to the true age of wild and captive manatees.

Development of an age-determination technique has allowed some insight into age-specific patterns of reproduction. Manatees have a long life span and low fecundity rates, but they may mature earlier in life than previously believed. According to this study, adult female manatees can produce only 1 offspring at approximately every 3.0 years, half of which will be females. The proportion of pregnant females in the population is variable from year to year, but when the information is pooled by two-year intervals, the mean annual pregnancy rate is 33.3%. As a general pattern, the proportion of reproducing animals is

lower in recently mature females and increases in older females. Reproductive information from one large female supports the conclusion that they may remain reproductively active into older ages. Age-specific fecundity remained relatively constant throughout life, on the order of 24%, and senescence was undetected. Most of the carcass sample is comprised of young manatees. If females in their prime reproductive years are being selectively removed from the population, the reproductive rates may present a change with time. Despite the increasing number of carcasses found over the years, no apparent changes have occurred in age at first maturation. Annual pregnancy rates may have increased since 1976, but no correlation can be made with population density due to lack of density estimates. If reproduction increases at a comparable or higher rate than mortality increases, there is less reason for concern; on the other hand, if reproduction eventually decreases because of fewer animals available to mate, then the problem intensifies.

Analysis of a large data set of carcasses with cause-of-death identified indicated that mortality takes a larger toll on younger manatees, but after attainment of sexual maturity, the rate of mortality decreases considerably and remains constant throughout the rest of life. With their low reproductive capacity, manatees could not persist without extreme longevity of adults. Collision with boats,

the most prominent identifiable cause of death among manatees, affects all ages indiscriminately.

A life table was built using the age-at-death data, and assuming a stationary population. A frequent criticism to this approach is that it is based on a sample of dead animals and does not necessarily represent the living population. However, all the assumptions involved were addressed and believed to have been closely approximated. In addition, the similarity in results from field studies provided additional support for its use. There will always be doubts about aspects of manatee biology, habitat needs, and population dynamics, not to mention the randomness inherent to nature, but we must use the existing knowledge to take actions designed to meet the primary objective of manatee recovery.

The methods above were developed for vital rates assumed to be constant. However, except for aging, all events in an organism's life (mortality, reproduction, disease) are stochastic (Lacy, in press), so the constancy of a life table erroneously implies an indefinite null rate of increase for Florida manatees. By improving speed and adding flexibility to the original life tables, computer simulation models provide more accurate predictions about population dynamics than life tables (Lacy, in press). Nevertheless, it is important to also recognize the limitations of as mere tools to be used to test assumptions,

choose alternative management strategies, and predict the outcome of a population under various circumstances, not as substitutes for extensive empirical biological studies of populations and their natural history (Laws, 1981).

In February 1992 the U.S. Fish and Wildlife Service and the Florida Department of Natural Resources convened a workshop to synthesize information, evaluate progress, and identify research needs on manatee population biology. Results from long-term longitudinal studies of known, living individuals in the field (O'Shea and Langtimm, in manuscript; Rathbun et al., in manuscript; Reid et al., 1992), salvaged dead animals (Hernandez et al., in manuscript; Marmontel, in manuscript) and captive studies (Odell et al., 1992) demonstrated convergence in estimates of demographic parameters. This suggests that age criteria, ages assigned to specimens, and reproductive and survivorship data I have developed are reasonably accurate, rendering the calculation of survivorship curves based on a collection of earbones of manatees found dead a valid and useful technique in population studies. As more data are gathered, survivorship curves may detect changes in population structure with time. Because environmental changes result in fluctuations in populations (Seber, 1973), the overall picture of the population provided by the carcass sample should be complemented with other methods of estimation. When integrated with results of aerial surveys,

field observations and counts and radiotelemetry studies, those data can provide more informative estimates of population trends.

Research Needs

Counts of growth layers in the periotic dome should continue to be used as an age-determination technique in Florida manatees, and future research should continue to explore relationships between these counts, bone-resorption levels, age, sex, and other possible factors such as diet, habitat, and reproductive events. Tetracycline-marked animals have helped validate the technique and the rate of deposition. Further tetracycline-marking of wild animals is encouraged, to help verify or clarify details for further assessment of environmental factors, stresses, and life-history events on growth-layer group formation and resorption in manatee bones. Guidelines and definitions for counts of seemingly double layers, traces of adhesion lines, and closely appressed growth-layer groups should be established. Precision in counting techniques and the effects of variability in counts due to observer skill and to sections within bones should be quantified, perhaps using an experimental design similar to that employed by Bjorge et al. (1990), and the results of such an experiment should be incorporated in standardization of procedure for future studies.

The nature of data collection does not allow the calculation of a population rate of increase through the use of the conventional life table. However, ages-at-death have been used to calculate population growth rate and survivorship schedules (Tait and Bunnell, 1980; Van Sickle et al., 1987). It is recommended that a follow-up of this study analyses the carcass data by the skull method (Van Sickle et al., 1987), as that approach is believed applicable to this kind of data and robust to sampling errors. The method requires an estimate of first-year survival, and consists of the calculation of rate of increase through the solution of a high order polynomial. The rate of increase may then be used to calculate survivorship values.

A suitable aerial census method has not so far been successfully developed (Lefebvre et al., 1992), but attempts at population estimation must be continued to obtain a careful quantitative estimate of current Florida manatee population or an index of trends in abundance. Appropriate monitoring of population parameters should be continued or developed, with the purpose of long-term management. This should include the carcass salvage program and field studies with direct observations of live animals. Annual probabilities of reproduction are more readily estimated than survival, so there is a need for a greater focus on estimating survival (along with compensatory responses, if

detected). An intensive sight-resight program based on individually-recognized animals has produced estimates for adults and should in the medium term provide data for juveniles. Effort should be directed toward areas most likely to produce valuable returns, such as the well studied populations of Crystal River and Blue Spring. The parameters thus obtained must be updated and put into refined population models specifically designed for manatees on a regular basis to provide managers with better projections. Repeating the population viability analysis (including estimated derived from the skull method noted above) with new data and under new scenarios will confirm or refute its predictive power, improve our understanding of the dynamics of the population and allow for adjustment of management options accordingly. Viability should be modeled using population parameters derived from studies of free-ranging manatees, and subpopulations where differences in parameters may be identified. It is also necessary to develop a methodology and programs to monitor the condition of essential manatee feeding habitats, and the total environmental impact of coastal development needs to be evaluated.

Management Implications/Recommendations

Results from the life-table and modeling approaches indicate that the Florida manatee population has a low rate

of increase; even if reproduction were to substantially exceed mortality, reproductive potential is naturally (physiologically) low, limited, and recovery after a major decline would be slow. Population viability analysis is a probability issue (Shaffer, 1981). The simulations do not predict with certainty what will happen to the Florida manatee, but project the most likely outcome of the population under the mean population parameters calculated from long-term data. The actual values may be higher, in which case the situation might be better than depicted, but the similarity between parameters presented at the 1992 Manatee Population Biology Workshop suggest that they are reasonably correct. It is more likely that some factors contributing to instability were not accounted for (i.e., genetics) and there is considerable uncertainty as to environmental conditions and coastal development in the future (e.g., food supply decimation, habitat degradation). The modeling of a stable population shows that variability in the population parameters, any additional stress, or a catastrophe might tip the balance towards a greater risk of extinction. Any degree of exploitation in the broad sense of the word would pose a threat to the population's maintenance. Therefore, management measures must be taken to ensure that the population has a safe margin of persistence. To maintain a viable manatee population with a non-negative net rate of increase, the main concern should

be to maximize the Florida manatee population's growth parameter r . This would improve prospects of and accelerate the process of recovery in case of a sudden decline.

Two alternatives can potentially improve r : an increase in the reproductive rate or a reduction in mortality. Short of massive and expensive captive breeding programs, there is practically no way to improve reproduction, but identifying and protecting undisturbed, sheltered sites used by manatees should secure favorable calving grounds. Enforcing and expanding present legislation on speed zones and limits, on the other hand, should minimize mortality. Manatees are able to respond to their environment and present a considerable flexibility to adjust to local long- and short-term habitat conditions (O'Shea and Ludlow, 1992). If habitat is protected, especially as related to reproduction and feeding, while reducing mortality levels, the population would become much less vulnerable to the other factors that are beyond the control of managers (demographic, environmental or catastrophic variation). However, there is no guarantee that habitat will be effectively protected, and there is no margin for error. Given the high incidence of boat-related mortality, and rapid growth in boat traffic and coastal development, the prognosis is not good. Only scenarios with low environmental variation, minimal effect of catastrophes and low mortality rates resulted in low probability of extinction.

The results from this study justify a greater emphasis in management efforts to minimize or mitigate mortality as a limiting factor in manatee dynamics. Thirteen counties are considered key to manatee dynamics, because of high use and risk of vessel-related mortality. As part of their growth management plans, boat speed regulatory zones have been developed for waterways of those counties, but so far adopted only in 8 of them; the remaining 5 counties must be urged to implement those measures. A few percentage points in adult survival can make a large difference, so it is necessary to reduce all identifiable sources of mortality, including collisions with large, slow-moving vessels, entrapment and drowning in culverts. Educational campaigns and the proposed boat operator registration should help increase awareness and reduce vessel-manatee collisions.

Although mortality is the most conspicuous factor now, we must manage with the long-term in mind to safeguard from extinction and lack of adaptation. Man can dramatically alter natural processes of habitats, including critical areas for manatees, required to sustain viable populations, and habitat alteration is likely to become more important in the future. The Marine Mammal Protection Act calls for the maintenance of health and stability of marine mammals and their ecosystem. Future coastal development will translate into degradation and loss of habitat, and reduction in the food base. Manatees are already under various levels of

human intervention: migration patterns have been altered by thermal discharges, freshwater and unnatural food are offered to manatees next to urban settings in various parts of the state, manatees have become accustomed to and interact closely with people in areas like Crystal River, and a rehabilitation program is a routine management activity for injured and rescued manatees. If beneficial in some aspects, these activities bring manatees in closer proximity to humans and consequently greater danger. Further interventions must be minimized by preventing loss of habitat, permitting continued movements along traditional travel routes, and increasing the network of sanctuaries and refuges.

The objective of the Florida manatee recovery plan is to establish "a viable, self-sustaining population of manatees on both the Atlantic and Gulf coasts". To obtain this, mortality factors and threats must be controlled or decreased, and habitats must be secured (U.S. Fish and Wildlife Service, 1989). Despite the apparent stability in manatee population growth, the uncertainty in the remaining parameters warrants the appropriateness and maintenance of the status of "endangered" as designated by the U.S. Fish and Wildlife Service. Populations of at least a few thousand manatees will be necessary to assure persistence into the future. Simulations projecting a high level of persistence but with a negligible mean final population size

will certainly not guarantee the necessary levels of genetic variability to assure evolution. Reduction in mortality levels must be secured to prevent the need of a metapopulation approach with human intervention (entailing moving animals around) or the very costly option of captive breeding.

Florida and Georgia probably contain the largest population of West Indian manatees. In all other parts of the range the species occurs in the low hundreds and is faced with threats of habitat alteration and local hunting. Manatees in the southeastern United States have the best chance of persisting into the future, but their protection and preservation may require political decisions and change in attitudes of part of the public.

APPENDIX A

BIOLOGICAL DATA FOR *TRICHECHUS MANATUS LATIROSTRIS*
OF UNKNOWN AGE USED IN THIS STUDY

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
CAS 23277	M-293	M	98.0	MAY 82	0	N	NONE
CAS 23278	M-260	F	317.0	JAN 82	15	N	MODER
CAS 23279	M-84-22	F	242.0	APR 84	1	N	NONE
CAS 23280	M-273	F	208.0	MAR 82	0	N	NONE
CAS 23281	M-82-20	M	258.0	FEB 82	2	N	NONE
CAS 23282	M-82-14	M	261.0	FEB 82	4	N	NONE
CAS 23283	M-81-15	M	297.0	FEB 81	13	N	LIGHT
CAS 23285	M-268	F	202.0	FEB 82	0	N	NONE
CAS 23286	M-334	F	245.0	MAY 83	1	N	NONE
CAS 23287	M-81-67	F	175.0	DEC 81	1	N	NONE
CAS 23288	M-83-20	F	304.0	MAY 83	16	N	MODER
CAS 23289	M-81-66	M	222.0	DEC 81	1	N	NONE
CAS 23290	M-82-03	F	181.0	JAN 82	1	N	NONE
CAS 23291	M-82-56	F	318.0	DEC 82	9	Y	MODER
CAS 23293	M-82-52	M	300.0	AUG 82	6	N	NONE
CIMNH 733	M-364	M	212.0	JAN 84	1	N	NONE
CIMNH 734	M-366	F	231.0	JAN 84	1	N	NONE
CIMNH 735	M-367	M	222.0	JAN 84	1	N	NONE
CIMNH 736	M-368	F	183.0	FEB 84	0	N	NONE
CIMNH 737	M-370	F	237.0	FEB 84	1	N	NONE
CIMNH 738	M-372	M	210.0	FEB 84	1	N	NONE
CIMNH 740	M-82-30	M	320.0	MAR 82	11	N	LIGHT
CIMNH 741	M-82-31	M	319.0	MAR 82	7	N	LIGHT
CIMNH 742	M-82-34	M	252.0	APR 82	4	N	NONE
CIMNH 743	M-82-39	F	367.0	MAY 82	19	Y	MODER
CIMNH 744	M-82-40	M	234.0	JUN 82	1	N	NONE
CIMNH 745	M-82-43	M	283.0	JUL 82	3	N	NONE
CIMNH 746	M-82-44	F	218.0	JUL 82	1	N	NONE
CIMNH 748	M-82-50	F	250.0	AUG 82	2	N	NONE
CIMNH 749	M-82-53	F	270.0	OCT 82	3	N	NONE
CIMNH 750	M-82-55	F	270.0	DEC 82	8	N	LIGHT
CIMNH 751	M-82-57	F	178.0	DEC 82	1	N	NONE
CM 77798	M-219	M	241.0	JAN 81	2	N	NONE
CM 77799	M-220	M	244.0	JAN 81	2	N	NONE
CM 77800	M-224	F	223.0	FEB 81	1	N	NONE
CM 77802	M-199	F	294.0	AUG 80	5	N	NONE
CM 77803	M-194	F	274.0	JUN 80	3	N	NONE
CM 77804	M-234	F	319.0	APR 81	8	N	MODER
CM 77805	M-197	M	277.0	JUL 80	8	N	LIGHT
CM 77806	M-201	F	304.0	SEP 80	5	N	NONE
CM 77807	M-189	M	261.0	MAR 80	2	N	NONE
CM 77808	M-230	F	290.0	MAR 81	3	N	NONE
CM 77809	M-240	F	199.0	MAY 81	0	N	NONE
CM 77810	M-237	M	290.0	MAY 81	8	N	LIGHT
CM 77811	M-223	M	271.0	FEB 81	7	N	LIGHT
CM 77812	M-226	F	259.0	MAR 81	2	N	NONE
CM 77813	M-196	F	316.0	JUL 80	11	N	LIGHT
CM 77814	M-212	F	272.0	NOV 80	2	N	NONE
CM 77815	M-222	F	265.0	JAN 81	3	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
CM 77816	M-209	M	299.0	OCT 80	4	N	NONE
CM 77817	M-235	M	318.0	MAY 81	25	N	MODER
LACM 72225	M-218	M	263.0	JAN 81	6	N	LIGHT
LACM 72226	M-225	F	325.0	FEB 81	28	Y	HEAVY
LACM 72227	M-229	M	309.0	MAR 81	37	Y	HEAVY
LACM 72228	M-239	M	263.0	MAY 81	3	N	NONE
LACM 72229	M-244	M	190.0	JUN 81	0	N	NONE
LACM 72230	M-246	M	304.0	JUL 81	15	N	MODER
LACM 72231	M-249	M	298.0	JUL 81	8	N	LIGHT
LACM 72232	M-250	F	248.0	AUG 81	3	N	NONE
LACM 72233	M-254	M	255.0	SEP 81	3	N	NONE
LACM 72234	M-255	M	314.0	NOV 81	18	N	MODER
LACM 72235	M-256	F	308.0	DEC 81	7	N	LIGHT
LACM 72236	M-259	F	282.0	JAN 82	6	N	LIGHT
LACM 72238	M-262	F	162.0	JAN 82	0	N	NONE
LACM 72239	M-266	F	225.0	FEB 82	2	N	NONE
LACM 72240	M-275	M	260.0	MAR 82	3	N	NONE
LACM 72244	M-292	M	276.0	APR 82	8	N	LIGHT
LACM 72246	M-328	F	275.0	APR 83	2	N	NONE
LACM 72249	M-343	F	155.0	JUL 83	0	N	NONE
LACM 72250	M-361	M	136.0	JAN 84	0	N	NONE
LACM 72252	M-81-16	M	212.0	FEB 81	1	N	NONE
LACM 72254	M-81-26	M	250.0	FEB 81	4	N	NONE
LACM 72256	M-81-59	M	231.0	SEP 81	2	N	NONE
LACM 72258	M-81-68	M	195.0	DEC 81	0	N	NONE
LACM 72259	M-82-02	F	335.0	JAN 82	8	N	LIGHT
LACM 72260	M-82-07	M	183.0	FEB 82	1	N	NONE
LACM 72261	M-82-10	F	277.0	FEB 82	5	N	LIGHT
LACM 72262	M-82-15	M	303.0	FEB 82	7	N	LIGHT
LACM 72263	M-82-18	F	318.0	FEB 82	19	Y	HEAVY
LACM 72266	M-82-24	F	286.0	FEB 82	7	N	NONE
LACM 72267	M-82-25	F	308.0	MAR 82	11	N	LIGHT
LACM 72268	M-82-29	M	288.0	MAR 82	11	N	LIGHT
LACM 72270	M-82-33	F	213.0	APR 82	1	N	NONE
LACM 72272	M-82-36	F	246.0	APR 82	4	N	NONE
MSB 50450	M-269	F	135.0	FEB 82	0	N	NONE
MSB 50454	M-81-08	F	151.0	JAN 81	0	N	NONE
MSB 50455	M-81-62	F	330.0	OCT 81	23	Y	HEAVY
MSB 50456	M-81-61	F	293.0	OCT 81	3	N	NONE
MSB 50457	M-81-49	F	275.0	MAY 81	7	N	LIGHT
MSB 50460	M-81-36	M	283.0	MAR 81	14	N	MODER
MSB 50461	M-81-05	F	259.0	JAN 81	5	N	NONE
MSB 50463	M-274	F	130.0	MAR 82	0	N	NONE
MSB 50464	M-81-38	F	307.0	MAR 81	12	N	MODER
MSB 50465	M-81-35	F	180.0	FEB 81	0	N	NONE
MSB 50466	M-81-60	M	335.0	SEP 81	25	Y	HEAVY
MSB 50467	M-81-34	F	265.0	FEB 81	10	N	MODER
MSB 50468	M-81-24	F	264.0	FEB 81	2	N	NONE
MSB 50469	M-81-44	M	286.0	APR 81	4	N	NONE
NCSM 3618	M-019	U	269.0	MAR 76	9	N	MODER
NCSM 4563	M-388	M	209.0	MAY 84	0	N	NONE
NCSM 4564	M-247	M	325.0	JUL 81	24	N	HEAVY
NCSM 4565	M-338	F	340.0	JUN 83	14	N	MODER
NCSM 4566	M-355	M	302.0	JAN 84	14	N	MODER
NCSM 4567	M-339	M	291.0	JUN 83	9	N	LIGHT
NCSM 4568	M-326	M	237.0	MAR 83	3	N	NONE
NCSM 4570	M-342	F	235.0	JUL 83	1	N	NONE
NCSM 4571	M-347	M	117.0	SEP 83	0	N	NONE
NCSM 4572	M-310	M	302.0	SEP 82	6	N	LIGHT
NCSM 4573	M-337	F	144.0	JUN 83	0	N	NONE
NCSM 4574	M-358	F	250.0	JAN 84	2	N	NONE
NCSM 4575	M-340	M	130.0	JUL 83	0	N	NONE
NCSM 4576	M-360	F	170.0	JAN 84	0	N	NONE
NCSM 4577	M-348	M	267.0	SEP 83	3	N	NONE
NCSM 5252	M-462	U	225.0	MAR 86	2	N	NONE
NCSM 5426	MSW-014	M	282.0	JAN 84	4	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
NCSM 5429	M-280	M	267.0	MAR 82	7	N	LIGHT
NCSM 5430	M-288	F	335.0	APR 82	16	N	MODER
NCSM 5431	M-322	M	272.0	FEB 83	3	N	NONE
NCSM 5432	M-333	M	220.0	APR 83	2	N	NONE
NCSM 5433	M-369	M	274.0	FEB 84	7	N	LIGHT
NCSM 5434	M-373	F	243.0	FEB 84	3	N	NONE
NCSM 5435	M-81-71	U	300.0	DEC 81	26	Y	HEAVY
NCSM 5436	M-82-01	F	190.0	JAN 82	1	N	NONE
NCSM 5437	M-82-51	F	290.0	AUG 82	3	N	NONE
NCSM 5438	M-83-02	M	284.0	JAN 83	5	N	NONE
NCSM 5439	M-83-05	F	139.0	FEB 83	0	N	NONE
NCSM 5440	M-83-10	F	188.0	MAR 83	0	N	NONE
NCSM 5441	M-83-13	M	283.0	MAR 83	9	N	LIGHT
NCSM 5442	M-83-14	M	308.0	MAR 83	17	N	LIGHT
NCSM 5443	M-83-17	M	227.0	APR 83	1	N	NONE
NCSM 5444	M-83-23	U	290.0	JUN 83	7	N	LIGHT
NCSM 5445	M-83-34	F	318.0	AUG 83	12	N	MODER
NCSM 5446	M-83-37	M	325.0	OCT 83	20	N	MODER
NCSM 5447	M-83-40	M	259.0	DEC 83	1	N	NONE
NCSM 5448	M-84-06	F	316.0	JAN 84	27	Y	HEAVY
NCSM 5449	M-84-18	M	300.0	APR 84	14	N	MODER
NCSM 5450	M-84-27	F	243.0	AUG 84	1	N	NONE
NCSM 6658	M-83-24	M	280.0	JUN 83	4	N	NONE
NCSM 6659	M-84-13	M	290.0	FEB 84	10	N	LIGHT
NM 259406		M	0.0	JAN 29	19	Y	HEAVY
NM 527899	M-094	F	147.5	NOV 77	0	N	NONE
NM 527900	M-095	F	334.4	DEC 77	9	N	LIGHT
NM 527901	M-096	M	324.0	DEC 77	14	N	LIGHT
NM 527903	M-098	M	264.0	JAN 78	6	N	LIGHT
NM 527905	M-100	F	272.0	JAN 78	13	N	MODER
NM 527911	M-107	F	245.0	FEB 78	2	N	NONE
NM 527912	M-108	M	274.0	MAR 78	6	N	LIGHT
NM 527913	M-109	M	167.0	MAR 78	0	N	NONE
NM 527916	M-112	M	300.0	APR 78	10	N	MODER
NM 527918	M-115	M	143.0	APR 78	0	N	NONE
NM 527920	M-117	M	351.0	MAY 78	21	Y	HEAVY
NM 527921	M-118	M	138.0	MAY 78	0	N	NONE
NM 527922	M-119	F	147.0	MAY 78	0	N	NONE
NM 527923	M-120	F	115.0	MAY 78	0	N	NONE
NM 527925	M-122	M	270.0	JUN 78	4	N	NONE
NM 527926	M-123	F	254.0	JUN 78	3	N	NONE
NM 527927	M-124	F	340.0	JUL 78	16	N	MODER
NM 530294	M-128	M	315.0	OCT 78	9	N	LIGHT
NM 530297	M-131	M	263.0	DEC 78	6	N	LIGHT
NM 530298	M-132	M	131.0	DEC 78	0	N	NONE
NM 530299	M-155	F	355.0	JUN 79	25	Y	HEAVY
NM 530300	M-75-05	M	142.0	APR 75	0	N	NONE
NM 530301	M-77-07	F	345.0	FEB 77	29	Y	MODER
NM 530302	M-77-09	M	173.0	FEB 77	0	N	NONE
NM 530303	M-77-16	M	267.0	MAY 77	2	N	NONE
NM 530304	M-77-20	F	229.0	JUN 77	1	N	NONE
NM 530305	M-77-21	M	295.0	JUN 77	7	N	LIGHT
NM 530306	M-77-22	F	345.0	JUN 77	22	Y	MODER
NM 530310	M-78-02	M	257.0	JAN 78	3	N	NONE
NM 530311	M-78-08	M	285.0	FEB 78	13	N	MODER
NM 530312	M-78-09	F	281.0	FEB 78	3	N	NONE
NM 530313	M-78-10	M	312.0	FEB 78	19	Y	HEAVY
NM 530314	M-78-11	F	222.0	FEB 78	2	N	NONE
NM 530315	M-78-15	M	291.0	FEB 78	11	N	LIGHT
NM 530316	M-78-16	F	275.0	FEB 78	10	N	MODER
NM 530318	M-78-20	M	214.0	MAR 78	2	N	NONE
NM 530320	M-78-33	U	190.0	AUG 78	1	N	NONE
NM 530321	M-198	F	322.0	AUG 80	9	N	LIGHT
NM 530322	M-78-39	F	300.0	OCT 78	12	N	LIGHT
NM 530324	M-78-42	M	255.0	NOV 78	2	N	NONE
NM 530325	M-78-44	M	230.0	DEC 78	1	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
NM 530327	M-79-05	M	195.0	FEB 79	1	N	NONE
NM 530328	M-79-06	F	224.0	FEB 79	1	N	NONE
NM 550329	M-276	M	296.0	MAR 82	10	N	NONE
NM 550421	M-331	F	375.0	APR 83	29	Y	LIGHT
NM 551656	M-133	F	258.0	JAN 79	6	N	HEAVY
NM 551657	M-134	M	273.0	JAN 79	7	N	LIGHT
NM 551658	M-135	M	133.0	FEB 79	0	N	NONE
NM 551660	M-137	M	228.0	FEB 79	3	N	NONE
NM 551663	M-140	M	307.0	MAR 79	17	N	NONE
NM 551666	M-144	M	175.0	APR 79	0	N	HEAVY
NM 551668	M-148	F	124.0	MAY 79	1	N	NONE
NM 551670	M-154	M	217.5	MAY 79	1	N	NONE
NM 551671	M-156	M	279.0	JUN 79	6	N	NONE
NM 551672	M-146	M	320.0	APR 79	13	Y	LIGHT
NM 551673	M-160	M	133.0	JUN 79	0	N	MODER
NM 551674	M-162	M	144.0	JUL 79	0	N	NONE
NM 551675	M-167	F	135.0	AUG 79	0	N	NONE
NM 551676	M-172	F	142.0	SEP 79	0	N	NONE
NM 551677	M-173	F	141.0	SEP 79	0	N	NONE
NM 551679	M-177	M	139.0	DEC 79	0	N	NONE
NM 551680	M-178	M	119.0	DEC 79	0	N	NONE
NM 551681	M-143	M	292.0	APR 79	8	N	NONE
NM 554180	M-179	F	230.0	JAN 80	2	N	NONE
NM NO #	M-82-13	M	219.0	FEB 82	1	N	NONE
NM NO #	MSU-011	F	241.0	JAN 84	2	N	NONE
OMNH	M-277	F	321.0	MAR 82	10	N	NONE
OMNH	M-305	M	307.0	AUG 82	10	N	LIGHT
OMNH	M-306	M	295.0	AUG 82	6	N	MODER
OMNH	M-308	M	237.0	AUG 82	2	N	LIGHT
OMNH 17189	M-296	M	265.0	MAY 82	3	N	NONE
OMNH 17190	M-302	F	242.0	JUL 82	2	N	NONE
OMNH 17192	M-313	F	251.0	OCT 82	1	N	NONE
OMNH 17194	M-321	F	245.0	FEB 83	1	N	NONE
OMNH 17195	M-323	F	199.0	FEB 83	0	N	NONE
OMNH 17196	M-324	M	236.0	MAR 83	3	N	NONE
OMNH 17198	M-330	M	230.0	APR 83	2	N	NONE
OMNH 17201	M-82-08	M	219.0	FEB 82	0	N	NONE
OMNH 17202	M-82-11	F	210.0	FEB 82	0	N	NONE
OMNH 17203	M-82-12	M	226.0	FEB 82	1	N	NONE
OMNH 17204	M-82-19	F	250.0	FEB 82	2	N	NONE
OMNH 17205	M-82-21	F	272.0	FEB 82	3	N	NONE
OSU 11710	M-279	M	227.0	MAR 82	2	N	NONE
OSU 11711	M-350	M	199.0	DEC 83	0	N	NONE
OSU 11713	M-83-15	F	308.0	MAR 83	8	N	NONE
OSU 11714	M-82-16	M	225.0	FEB 82	1	N	LIGHT
OSU 11716	M-392	M	139.0	JUL 84	0	N	NONE
OSU 11717	M-332	F	113.0	APR 83	0	N	NONE
OSU 11718	M-216	F	255.0	JAN 81	6	N	NONE
OSU 11720	M-83-08	M	297.0	FEB 83	10	N	NONE
OSU 11721	M-263	M	205.0	JAN 82	2	N	LIGHT
OSU 11722	M-264	F	225.0	JAN 82	1	N	NONE
OSU 11723	M-217	M	252.0	JAN 81	3	N	NONE
OSU 11724	M-294	F	231.0	MAY 82	1	N	NONE
OSU 11726	M-384	F	116.0	APR 84	0	N	NONE
OSU 11727	M-81-65	M	215.0	DEC 81	1	N	NONE
OSU 11728	M-82-09	F	324.0	FEB 82	11	N	NONE
OSU 11729	M-81-63	M	346.0	DEC 81	25	Y	MODER
UF 08481		U	0.0		0	N	HEAVY
UF 13257		U	0.0	APR 74	0	N	NONE
UF 13405		U	0.0	JUN 81	21	Y	NONE
UF 13553	M-015	M	175.0	DEC 75	0	N	HEAVY
UF 13571	M-018	F	203.0	MAR 76	0	N	NONE
UF 13877	M-036	F	351.0	NOV 76	22	Y	NONE
UF 13889	M-040	M	0.0	JAN 77	0	N	HEAVY
UF 13892	M-043	F	190.0	JAN 77	0	N	NONE
UF 13894	M-045	F	157.5	JAN 77	0	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
UF 13900	M-049	F	157.0	FEB 77	0	N	NONE
UF 13920	M-069	F	231.0	FEB 77	2	N	NONE
UF 13923	M-072	M	192.5	MAR 77	0	N	NONE
UF 13988	M-077	M	211.5	MAR 77	0	N	NONE
UF 13991	M-078	U	228.6	APR 77	1	N	NONE
UF 14207	M-091	F	135.0	OCT 77	0	N	NONE
UF 14815	M-083	M	129.5	MAY 77	0	N	NONE
UF 15110	M-147	F	245.0	APR 79	3	N	NONE
UF 15111	M-149	F	263.0	MAY 79	3	N	NONE
UF 15112	M-150	F	289.0	MAY 79	5	N	LIGHT
UF 15113	M-151	F	0.0	MAY 79	5	N	NONE
UF 15114	M-153	M	256.0	MAY 79	4	N	NONE
UF 15115	M-157	F	362.0	JUN 79	59	Y	HEAVY
UF 15116	M-158	F	244.0	JUN 79	2	N	NONE
UF 15118	M-161	F	152.0	JUL 79	0	N	NONE
UF 15120	M-164	M	305.0	JUL 79	7	N	NONE
UF 15121	M-165	M	300.0	JUL 79	29	N	MODER
UF 15122	M-166	M	277.0	AUG 79	3	N	NONE
UF 15123	M-168	F	280.0	AUG 79	3	N	NONE
UF 15125	M-170	M	204.0	SEP 79	1	N	NONE
UF 15126	M-171	M	192.0	SEP 79	1	N	NONE
UF 15127	M-174	M	273.0	OCT 79	5	N	NONE
UF 15128	M-175	M	145.0	OCT 79	0	N	NONE
UF 15129	M-180	M	207.0	JAN 80	1	N	NONE
UF 15130	M-182	F	242.0	JAN 80	3	N	LIGHT
UF 15132	M-188	M	152.0	MAR 80	0	N	NONE
UF 15133	M-190	F	110.0	APR 80	0	N	NONE
UF 15134	M-191	F	103.0	APR 80	0	N	NONE
UF 15135	M-193	F	139.0	MAY 80	0	N	NONE
UF 15136	M-213	M	117.0	DEC 80	0	N	NONE
UF 15137	M-214	F	132.0	DEC 80	0	N	NONE
UF 15138	M-221	M	133.0	JAN 81	0	N	NONE
UF 15139	M-228	F	119.0	MAR 81	0	N	NONE
UF 15140	M-231	M	136.0	MAR 81	0	N	NONE
UF 15141	M-232	M	190.0	APR 81	0	N	NONE
UF 15142	M-233	M	145.0	APR 81	0	N	NONE
UF 15143	M-238	F	143.0	MAY 81	0	N	NONE
UF 15144	M-241	F	143.0	MAY 81	0	N	NONE
UF 15145	M-243	M	126.0	MAY 81	0	N	NONE
UF 15146	M-245	F	117.0	JUN 81	0	N	NONE
UF 15150	M-75-08	M	100.0	MAY 75	0	N	NONE
UF 15151	M-80-14	F	320.0	MAY 80	8	N	MODER
UF 15152	M-75-10	F	117.0	JUN 75	0	N	NONE
UF 15157	M-77-39	F	127.0	NOV 77	0	N	NONE
UF 15159	M-78-06	F	225.0	FEB 78	1	N	NONE
UF 15160	M-78-07	F	315.0	JAN 78	12	Y	HEAVY
UF 15161	M-78-12	F	194.0	FEB 78	1	N	NONE
UF 15162	M-78-22	M	217.0	MAR 78	1	N	NONE
UF 15163	M-78-37	M	167.0	OCT 78	0	N	NONE
UF 15165	M-79-01	M	172.0	JAN 79	1	N	NONE
UF 15169	M-79-08	M	303.0	FEB 79	13	N	LIGHT
UF 15170	M-79-09	M	225.0	FEB 79	2	N	NONE
UF 15171	M-79-17	F	229.0	AUG 79	1	N	NONE
UF 15172	M-79-18	M	273.0	AUG 79	19	N	MODER
UF 15173	M-79-20	F	326.0	SEP 79	11	N	MODER
UF 15174	M-79-21	F	295.0	SEP 79	9	N	LIGHT
UF 15175	M-79-22	U	0.0	OCT 79	8	N	LIGHT
UF 15176	M-79-24	M	235.0	NOV 79	1	N	NONE
UF 15177	M-79-25	F	298.0	NOV 79	6	N	NONE
UF 15178	M-79-26	M	205.0	DEC 79	1	N	NONE
UF 15179	M-80-01	M	129.0	JAN 80	0	N	NONE
UF 15180	M-80-02	M	270.0	JAN 80	5	N	NONE
UF 15181	M-80-03	M	134.0	JAN 80	0	N	NONE
UF 15182	M-80-04	F	200.0	JAN 80	0	N	NONE
UF 15184	M-80-06	M	291.0	FEB 80	7	N	NONE
UF 15185	M-80-07	F	232.0	FEB 80	2	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
UF 15186	M-80-09	F	335.0	FEB 80	19	Y	HEAVY
UF 15187	M-80-10	M	324.0	MAR 80	12	N	LIGHT
UF 15188	M-80-11	U	195.0	MAR 80	0	N	NONE
UF 15189	M-80-12	M	190.0	MAR 80	1	N	NONE
UF 15190	M-80-13	F	310.0	APR 80	7	N	MODER
UF 15192	M-80-15	M	310.0	MAY 80	14	N	MODER
UF 15193	M-80-16	F	311.0	JUN 80	14	N	HEAVY
UF 15196	M-80-18	M	305.0	JUL 80	6	N	NONE
UF 15197	M-80-19	M	128.0	AUG 80	0	N	NONE
UF 15198	M-80-20	F	130.0	AUG 80	0	N	NONE
UF 15199	M-80-21	F	225.0	AUG 80	1	N	NONE
UF 15200	M-80-22	U	300.0	SEP 80	16	Y	HEAVY
UF 15202	M-80-24	M	305.0	OCT 80	16	N	MODER
UF 15203	M-80-25	M	287.0	NOV 80	10	N	LIGHT
UF 15204	M-80-26	M	137.5	NOV 80	0	N	NONE
UF 15206	M-81-01	F	204.0	JAN 81	0	N	NONE
UF 15207	M-81-03	M	204.0	JAN 81	0	N	NONE
UF 15208	M-81-04	F	192.0	JAN 81	0	N	NONE
UF 15209	M-81-10	M	182.0	JAN 81	0	N	NONE
UF 15210	M-81-43	M	152.0	MAR 81	0	N	NONE
UF 15211	M-81-48	M	110.0	APR 81	0	N	NONE
UF 15215	M-81-53	M	282.0	JUN 81	10	N	NONE
UF 15216	M-81-54	M	139.0	JUN 81	0	N	NONE
UF 15217	M-81-55	U	105.0	JUL 81	0	N	NONE
UF 18790		M	260.0	MAR 68	6	N	LIGHT
UF 19134	M-81-56	F	260.0	JUL 81	3	N	NONE
UF 19135	M-81-57	F	345.0	JUL 81	22	Y	HEAVY
UF 20592	M-379	M	105.0	MAR 84	0	N	NONE
UF 20594	M-412	F	250.0	NOV 84	2	N	NONE
UF 20595	M-413	M	260.0	NOV 84	2	N	NONE
UF 20596	M-414	M	242.0	NOV 84	2	N	NONE
UF 20597	M-415	M	145.0	DEC 84	0	N	NONE
UF 20598	M-423	F	277.0	FEB 85	3	N	NONE
UF 20599	M-428	M	252.0	FEB 85	9	N	NONE
UF 20600	M-432	M	204.0	FEB 85	1	N	NONE
UF 20601	M-435	F	280.0	MAR 85	10	N	MODER
UF 20602	M-444	M	213.0	APR 85	1	N	NONE
UF 20603	MSW-001	M	166.0	NOV 83	0	N	NONE
UF 20604	MSW-009	M	168.0	JAN 84	0	N	NONE
UF 20605	MSW-021	M	140.0	APR 84	0	N	NONE
UF 20606	MSW-024	M	190.5	MAY 84	0	N	NONE
UF 20607	M-83-36	F	123.0	OCT 83	0	N	NONE
UF 20608	M-84-14	F	344.0	FEB 84	22	Y	HEAVY
UF 20609	M-84-30	M	320.0	NOV 84	24	Y	HEAVY
UF 22380	M-84-33	F	144.0	DEC 84	0	N	NONE
UF 24953	M-270	F	295.0	FEB 82	9	N	MODER
UF 24954	M-299	M	318.0	MAY 82	14	N	MODER
UF 24955	M-300	M	356.0	JUN 82	18	Y	HEAVY
UF 24956	M-307	F	300.0	AUG 82	10	N	LIGHT
UF 24958	M-318	M	326.0	DEC 82	16	N	MODER
UF 24959	M-336	F	352.0	JUN 83	24	Y	MODER
UF 24960	M-346	F	300.0	AUG 83	5	N	LIGHT
UF 24961	M-349	M	330.0	OCT 83	17	Y	HEAVY
UF 24962	M-352	F	272.0	DEC 83	3	N	NONE
UF 24963	M-353	M	292.0	DEC 83	14	N	LIGHT
UF 24964	M-356	F	303.0	JAN 84	9	N	LIGHT
UF 24965	M-365	M	276.0	JAN 84	8	N	LIGHT
UF 24966	M-371	F	302.0	FEB 84	10	N	LIGHT
UF 24967	M-374	F	313.0	FEB 84	12	N	MODER
UF 24968	M-375	M	282.0	FEB 84	16	N	LIGHT
UF 24969	M-376	F	327.0	MAR 84	21	Y	HEAVY
UF 24970	M-381	M	301.0	MAR 84	9	N	MODER
UF 24972	M-389	F	313.0	JUN 84	7	N	LIGHT
UF 24975	M-391	M	303.0	JUL 84	12	N	LIGHT
UF 24977	M-396	F	310.0	JUL 84	8	N	LIGHT
UF 24978	M-398	F	230.0	JUL 84	4	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
UF 24980	M-401	M	310.0	AUG 84	10	N	LIGHT
UF 24985	M-410	F	142.0	NOV 84	0	N	NONE
UF 24988	M-417	M	225.0	JAN 85	2	N	NONE
UF 24989	M-422	F	375.0	FEB 85	28	Y	HEAVY
UF 24990	M-425	F	205.0	FEB 85	0	N	NONE
UF 24991	M-426	M	295.0	FEB 85	13	N	MODER
UF 24993	M-439	F	225.0	MAR 85	2	N	NONE
UF 24996	M-445	M	130.0	APR 85	0	N	NONE
UF 24998	M-448	M	241.0	APR 85	1	N	NONE
UF 25000	M-450	M	325.0	APR 85	15	N	MODER
UF 25001	M-452	F	220.0	MAY 85	1	N	NONE
UF 25003	M-456	M	307.0	JUN 85	4	N	NONE
UF 25005	M-457	F	325.0	JUN 85	8	N	LIGHT
UF 25006	M-458	M	295.0	JUN 85	24	Y	HEAVY
UF 25010	M-74-04	F	290.0	NOV 74	6	N	NONE
UF 25011	M-75-01	M	305.0	JAN 75	16	N	MODER
UF 25012	M-75-13	M	225.0	DEC 75	2	N	NONE
UF 25013	M-75-14	F	236.0	DEC 75	1	N	NONE
UF 25014	M-76-05	F	352.0	MAR 76	22	Y	MODER
UF 25015	M-76-07	F	316.0	MAR 76	9	N	LIGHT
UF 25017	M-76-16	M	343.0	JUL 76	8	N	LIGHT
UF 25018	M-76-19	F	335.0	AUG 76	18	N	MODER
UF 25019	M-76-21	F	285.0	SEP 76	4	N	NONE
UF 25020	M-76-23	F	350.0	OCT 76	19	N	MODER
UF 25021	M-76-29	F	335.0	NOV 76	12	N	MODER
UF 25022	M-77-14	M	162.0	MAR 77	0	N	NONE
UF 25023	M-77-15	F	216.0	MAR 77	2	N	NONE
UF 25024	M-77-31	M	295.0	OCT 77	5	N	NONE
UF 25025	M-77-33	F	245.0	OCT 77	2	N	NONE
UF 25026	M-77-34	F	310.0	OCT 77	15	N	MODER
UF 25027	M-77-35	F	246.0	NOV 77	1	N	NONE
UF 25028	M-77-36	F	300.0	NOV 77	10	N	LIGHT
UF 25029	M-77-38	M	252.0	NOV 77	2	N	NONE
UF 25030	M-77-41	M	329.0	NOV 77	29	Y	MODER
UF 25031	M-77-42	M	188.0	MAR 77	1	N	NONE
UF 25032	M-78-13	M	279.0	FEB 78	4	N	NONE
UF 25033	M-78-14	U	0.0	FEB 78	11	N	LIGHT
UF 25034	M-78-27	F	260.0	MAY 78	1	N	NONE
UF 25035	M-78-32	M	285.0	JUN 78	3	N	NONE
UF 25036	M-78-36	F	360.0	SEP 78	16	N	MODER
UF 25037	M-79-10	F	260.0	MAR 79	2	N	NONE
UF 25038	M-79-23	M	263.0	OCT 79	6	N	LIGHT
UF 25039	M-82-05	F	302.0	JAN 82	7	N	LIGHT
UF 25040	M-82-06	F	381.0	FEB 82	25	Y	HEAVY
UF 25041	M-82-17	F	283.0	FEB 82	5	N	LIGHT
UF 25042	M-82-26	F	294.0	MAR 82	3	N	NONE
UF 25044	M-82-45	U	275.0	JUL 82	18	Y	MODER
UF 25045	M-82-47	U	335.0	AUG 82	24	Y	HEAVY
UF 25046	M-82-48	F	302.0	AUG 82	8	N	MODER
UF 25047	M-83-03	F	350.0	FEB 83	19	Y	MODER
UF 25048	M-83-04	M	249.0	FEB 83	6	N	LIGHT
UF 25049	M-83-06	M	220.0	FEB 83	1	N	NONE
UF 25050	M-83-07	M	197.0	FEB 83	0	N	NONE
UF 25051	M-83-09	M	207.0	MAR 83	2	N	NONE
UF 25054	M-83-27	M	317.0	JUN 83	11	N	LIGHT
UF 25055	M-83-28	F	369.0	JUN 83	25	Y	HEAVY
UF 25058	M-83-32	U	300.0	JUL 83	25	Y	HEAVY
UF 25059	M-83-33	U	258.0	AUG 83	6	N	NONE
UF 25061	M-83-38	F	351.0	OCT 83	19	Y	MODER
UF 25062	M-83-39	F	315.0	NOV 83	13	N	MODER
UF 25063	M-84-01	F	345.0	DEC 83	17	Y	HEAVY
UF 25064	M-84-02	M	288.0	JAN 84	10	N	LIGHT
UF 25066	M-84-05	M	242.0	JAN 84	2	N	NONE
UF 25067	M-84-15	F	172.0	FEB 84	0	N	NONE
UF 25068	M-84-16	M	252.0	MAR 84	3	N	NONE
UF 25069	M-84-19	U	250.0	APR 84	1	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
UF 25071	M-84-25	M	289.0	JUL 84	8	N	LIGHT
UF 25072	M-84-26	F	168.0	AUG 84	0	N	NONE
UF 25073	M-84-28	M	291.0	SEP 84	8	N	LIGHT
UF 25074	M-84-31	M	215.0	DEC 84	2	N	NONE
UF 25075	M-84-32	F	194.0	DEC 84	1	N	NONE
UF 25076	M-85-02	F	173.0	JAN 85	0	N	NONE
UF 25077	M-85-03	F	191.0	JAN 85	1	N	NONE
UF 25078	M-85-05	M	201.0	FEB 85	0	N	NONE
UF 25079	M-85-06	F	223.0	FEB 85	1	N	NONE
UF 25080	M-85-07	M	183.0	FEB 85	0	N	NONE
UF 25081	M-85-10	F	321.0	FEB 85	17	N	MODER
UF 25082	M-85-11	M	215.0	FEB 85	1	N	NONE
UNBM 34504	MSU-015	M	269.0	FEB 84	4	N	NONE
UNBM 34506	M-83-16	F	202.0	MAR 83	2	N	NONE
UNBM 34507	M-84-07	M	242.0	JAN 84	2	N	NONE
UNBM 34508	M-84-08	F	225.0	JAN 84	1	N	NONE
UNBM 34509	M-84-11	M	248.0	FEB 84	2	N	NONE
UNBM 34510	M-84-12	F	258.0	FEB 84	2	N	NONE
UNBM 34511	M-84-17	M	264.0	MAR 84	6	N	NONE
UNBM 34512	M-84-23	M	221.0	MAY 84	1	N	NONE
UNBM 34514	M-208	M	297.0	OCT 80	8	N	LIGHT
UNBM 34515	M-286	M	288.0	APR 82	6	N	LIGHT
UNBM 34517	M-354	F	250.0	NOV 83	7	N	LIGHT
UNBM 34518	M-362	F	222.0	JAN 84	1	N	NONE
UNBM 34519	M-377	M	246.0	MAR 84	4	N	NONE
UNBM 34520	M-383	F	267.0	APR 84	1	N	NONE
UNBM 34521	M-397	M	290.0	AUG 84	13	N	MODER
UNBM 34522	M-395	F	310.0	JUL 84	11	N	MODER
	KDL-8702	M	190.0	JAN 87	0	N	NONE
	KDL-8704	M	238.0	FEB 87	2	N	NONE
	KDL-8709	M	248.0	FEB 87	3	N	NONE
	KDL-8710	F	223.0	MAR 87	1	N	NONE
	KDL-8712	F	118.0	MAR 87	0	N	NONE
	KDL-8717	F	170.0	APR 87	0	N	NONE
	KDL-8719	M	149.0	APR 87	0	N	NONE
	KDL-8720	F	170.0	APR 87	1	N	NONE
	KDL-8721	M	329.0	MAY 87	28	N	MODER
	KDL-8722	M	221.0	MAY 87	1	N	NONE
	KDL-8723	M	123.0	MAY 87	0	N	NONE
	KDL-8724	M	135.0	MAY 87	0	N	NONE
	KDL-8725	M	149.0	MAY 87	0	N	NONE
	KDL-8726	M	144.0	MAY 87	0	N	NONE
	KDL-8727	M	149.0	MAY 87	0	N	NONE
	KDL-8728	F	256.0	JUN 87	2	N	NONE
	KDL-8734	M	236.0	JUL 87	1	N	NONE
	KDL-8738	M	44.0	JUL 87	0	N	NONE
	KDL-8739	M	275.0	AUG 87	4	N	NONE
	KDL-8741	U	342.0	AUG 87	22	Y	HEAVY
	KDL-8743	F	140.0	AUG 87	0	N	NONE
	KDL-8745	F	380.0	SEP 87	39	Y	HEAVY
	KDL-8747	F	315.0	SEP 87	8	N	LIGHT
	KDL-8752	M	308.0	DEC 87	7	N	LIGHT
	KDL-8754	F	320.0	DEC 87	12	N	MODER
	KDL-8757	F	233.0	DEC 87	1	N	NONE
	KDL-8802	F	242.0	JAN 88	2	N	NONE
	KDL-8803	M	228.0	JAN 88	1	N	NONE
	KDL-8805	M	210.0	FEB 88	1	N	NONE
	KDL-8806	F	201.0	FEB 88	1	N	NONE
	KDL-8807	F	295.0	FEB 88	7	N	LIGHT
	KDL-8810	F	160.0	FEB 88	0	N	NONE
	KDL-8813	F	203.0	FEB 88	1	N	NONE
	KDL-8814	M	294.0	FEB 88	10	N	LIGHT
	KDL-8815	M	343.0	FEB 88	16	N	MODER
	KDL-8816	M	275.0	MAR 88	8	N	LIGHT
	KDL-8818	F	165.0	MAR 88	0	N	NONE
	KDL-8820	M	262.0	MAR 88	1	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
KDL-8821		F	315.0	MAR 88	10	N	LIGHT
KDL-8825		M	222.0	APR 88	1	N	NONE
KDL-8826		F	316.0	APR 88	9	N	LIGHT
KDL-8830		M	177.0	APR 88	1	N	NONE
KDL-8835		M	240.0	MAY 88	2	N	NONE
KDL-8837		F	111.0	MAY 88	0	N	NONE
KDL-8839		M	320.0	JUN 88	18	N	MODER
KDL-8840		M	256.0	JUN 88	4	N	LIGHT
KDL-8841		F	333.0	JUN 88	10	N	MODER
KDL-8842		F	275.0	JUN 88	6	N	LIGHT
KDL-8843		M	263.0	JUN 88	2	N	NONE
KDL-8847		F	345.0	JUL 88	24	Y	HEAVY
KDL-8848		M	305.0	JUL 88	7	N	LIGHT
KDL-8849		F	285.0	AUG 88	3	N	NONE
KDL-8850		M	291.0	AUG 88	6	N	NONE
KDL-8851		M	168.0	AUG 88	0	N	NONE
KDL-8852		F	283.0	AUG 88	3	N	NONE
KDL-8854		F	272.0	SEP 88	2	N	NONE
KDL-8855		F	325.0	SEP 88	11	N	MODER
KDL-8856		M	245.0	SEP 88	1	N	NONE
KDL-8857		F	290.0	SEP 88	16	N	LIGHT
KDL-8858		M	279.0	OCT 88	3	N	NONE
KDL-8859		M	305.0	OCT 88	13	N	MODER
KDL-8860		M	275.0	OCT 88	6	N	NONE
KDL-8862		F	268.0	NOV 88	2	N	NONE
KDL-8863		M	220.0	NOV 88	2	N	NONE
KDL-8864		M	280.0	DEC 88	7	N	NONE
KDL-8865		F	315.0	DEC 88	17	N	MODER
KDL-8866		M	182.0	DEC 88	0	N	NONE
KDL-8867		F	332.0	DEC 88	35	Y	HEAVY
KDL-8868		M	160.0	DEC 88	0	N	NONE
KDL-8901		F	230.0	JAN 89	1	N	NONE
KDL-8902		M	190.0	JAN 89	1	N	NONE
KDL-8903		F	178.0	JAN 89	0	N	NONE
KDL-8904		M	205.0	JAN 89	1	N	NONE
KDL-8905		M	162.0	JAN 89	0	N	NONE
KDL-8907		M	240.0	FEB 89	5	N	NONE
KDL-8908		F	231.0	FEB 89	1	N	NONE
KDL-8910		M	218.0	MAR 89	2	N	NONE
KDL-8915		M	310.0	MAR 89	14	N	LIGHT
KDL-8917		M	291.0	APR 89	8	N	LIGHT
KDL-8918		F	316.0	APR 89	8	N	LIGHT
KDL-8921		F	342.0	APR 89	16	N	MODER
KDL-8922		F	321.0	APR 89	14	N	MODER
KDL-8923		F	294.0	APR 89	4	N	NONE
KDL-8924		M	323.0	APR 89	20	N	MODER
KDL-8925		M	285.0	MAY 89	5	N	NONE
KDL-8927		M	308.0	MAY 89	7	N	LIGHT
KDL-8928		U	310.0	MAY 89	20	N	MODER
KDL-8929		M	343.0	MAY 89	21	N	MODER
KDL-8930		M	286.0	MAY 89	4	N	NONE
KDL-8931		F	296.0	MAY 89	6	N	LIGHT
KDL-8932		M	320.0	MAY 89	9	N	MODER
KDL-8933		M	253.0	MAY 89	5	N	NONE
KDL-8936		F	322.0	MAY 89	10	N	MODER
KDL-8937		F	340.0	JUN 89	12	N	MODER
KDL-8938		M	241.0	JUN 89	2	N	NONE
KDL-8941		M	288.0	JUL 89	6	N	MODER
KDL-8947		F	327.0	SEP 89	19	Y	MODER
M-195		F	276.0	JUN 80	3	N	NONE
M-320		M	185.0	FEB 83	1	N	NONE
M-429		M	260.0	FEB 85	6	N	LIGHT
M-430		M	310.0	FEB 85	11	N	MODER
M-431		F	282.0	FEB 85	8	N	LIGHT
M-74-03		M	305.0	AUG 74	17	N	LIGHT
M-75-02		F	295.0	FEB 75	4	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
M-75-03		M	260.0	FEB 75	2	N	NONE
M-75-06		M	306.0	MAY 75	7	N	NONE
M-76-04		M	130.0	MAR 76	0	N	NONE
M-76-18		F	135.0	JUL 76	0	N	NONE
M-76-22		F	276.0	OCT 76	6	N	LIGHT
M-76-28		M	278.0	NOV 76	7	N	LIGHT
M-76-30		M	181.0	DEC 76	0	N	NONE
M-76-32		M	104.5	JUL 76	0	N	NONE
M-77-02		F	200.0	JAN 77	2	N	NONE
M-77-04		F	220.0	JAN 77	1	N	NONE
M-77-18		F	290.0	MAY 77	9	N	LIGHT
M-77-24		F	350.0	JUL 77	35	Y	HEAVY
M-77-37		F	143.0	NOV 77	0	N	NONE
M-78-01		M	178.0	JAN 78	0	N	NONE
M-78-05		U	0.0	JAN 78	1	N	NONE
M-78-21		U	300.0	MAR 78	20	Y	HEAVY
M-78-24		M	204.0	MAR 78	2	N	NONE
M-78-25		U	350.0	MAR 78	8	N	LIGHT
M-78-26		U	200.0	APR 78	2	N	NONE
M-78-29		M	292.0	JUN 78	10	N	LIGHT
M-78-31		U	0.0	JUN 78	27	Y	HEAVY
M-78-34		F	387.0	AUG 78	26	Y	HEAVY
M-78-38		M	136.0	OCT 78	0	N	NONE
M-79-11		F	291.0	MAY 79	8	N	MODER
M-79-12		F	331.0	MAY 79	28	Y	HEAVY
M-79-15		M	287.0	MAY 79	11	N	LIGHT
M-79-19		M	296.0	SEP 79	8	N	LIGHT
M-84-24		M	325.0	JUN 84	24	Y	HEAVY
M-84-34		F	268.0	DEC 84	2	N	NONE
M-85-04		F	170.0	JAN 85	0	N	NONE
M-85-08		F	305.0	FEB 85	12	N	MODER
M-85-17		F	124.0	JUL 85	0	N	NONE
M-85-19		U	205.0	SEP 85	0	N	NONE
M-85-22		F	120.0	OCT 85	0	N	NONE
M-85-24		M	203.0	NOV 85	1	N	NONE
M-85-25		F	350.0	DEC 85	21	Y	HEAVY
M-85-26		M	282.0	DEC 85	3	N	NONE
M-85-27		F	332.0	DEC 85	11	N	MODER
M-86-01		M	206.0	JAN 86	3	N	NONE
M-86-03		F	145.0	JAN 86	0	N	NONE
M-86-04		F	239.0	JAN 86	2	N	NONE
M-86-05		M	305.0	JAN 86	14	N	LIGHT
M-86-07		F	145.0	JAN 86	0	N	NONE
M-86-08		F	208.0	JAN 86	1	N	NONE
M-86-09		M	177.0	JAN 86	1	N	NONE
M-86-11		F	262.0	MAR 86	1	N	NONE
M-86-12		F	288.0	MAR 86	6	N	LIGHT
M-86-13		M	195.0	MAR 86	1	N	NONE
M-86-14		F	362.0	MAR 86	16	N	HEAVY
M-86-16		M	116.0	MAY 86	0	N	NONE
M-86-17		M	285.0	MAY 86	9	N	LIGHT
M-86-20		M	310.0	AUG 86	10	N	LIGHT
M-86-21		M	304.0	OCT 86	24	Y	MODER
M-86-22		F	299.0	OCT 86	12	N	LIGHT
M-86-23		F	136.0	NOV 86	0	N	NONE
M-86-26		F	389.0	NOV 86	29	Y	HEAVY
M-86-27		F	200.0	DEC 86	0	N	NONE
M-86-28		F	227.0	DEC 86	1	N	NONE
M-87-05		M	162.0	FEB 87	0	N	NONE
M-87-06		M	216.0	FEB 87	1	N	NONE
M-87-07		M	222.0	FEB 87	3	N	NONE
M-87-08		F	225.0	FEB 87	1	N	NONE
M-87-10		M	314.0	APR 87	12	N	MODER
M-87-11		F	329.0	MAY 87	7	N	LIGHT
M-87-13		M	310.0	JUL 87	22	Y	HEAVY
M-87-14		M	337.0	JUL 87	11	N	LIGHT

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
M-87-18		F	214.0	NOV 87	1	N	NONE
MJAV-8802		M	300.0	JUL 88	12	N	LIGHT
MJAV-8920		F	347.0	SEP 89	15	N	MODER
MJAV-8922		F	355.0	NOV 89	24	Y	HEAVY
MJAV-8923		M	315.0	NOV 89	21	Y	MODER
MJAV-8924		F	180.0	NOV 89	0	N	NONE
MJAV-8925		F	180.0	DEC 89	1	N	NONE
MJAV-8926		F	296.0	DEC 89	6	N	MODER
MJAV-8927		M	282.0	DEC 89	4	N	NONE
MJAV-8928		M	220.0	DEC 89	1	N	NONE
MJAV-8929		M	246.0	DEC 89	2	N	NONE
MJAV-9001		M	218.0	JAN 90	1	N	NONE
MJAV-9002		F	207.0	JAN 90	1	N	NONE
MJAV-9003		F	200.0	JAN 90	0	N	NONE
MJAV-9004		F	154.0	JAN 90	0	N	NONE
MJAV-9005		M	260.0	JAN 90	2	N	NONE
MJAV-9006		F	220.0	JAN 90	2	N	NONE
MJAV-9007		F	310.0	JAN 90	12	N	LIGHT
MJAV-9008		M	307.0	JAN 90	11	N	LIGHT
MJAV-9010		M	222.0	JAN 90	1	N	NONE
MJAV-9011		M	326.0	JAN 90	18	Y	MODER
MJAV-9012		F	243.0	JAN 90	3	N	NONE
MJAV-9013		F	238.0	JAN 90	2	N	NONE
MJAV-9014		F	269.0	JAN 90	2	N	NONE
MJAV-9015		M	210.0	JAN 90	1	N	NONE
MJAV-9016		M	211.0	JAN 90	2	N	NONE
MJAV-9017		M	292.0	FEB 90	5	N	NONE
MJAV-9018		M	273.0	FEB 90	7	N	LIGHT
MJAV-9019		M	277.0	FEB 90	7	N	LIGHT
MJAV-9021		M	316.0	FEB 90	13	N	LIGHT
MJAV-9022		M	236.0	MAR 90	2	N	NONE
MJAV-9025		M	276.0	MAY 90	5	N	NONE
MJAV-9026		F	319.0	MAY 90	11	N	LIGHT
MJAV-9028		F	279.0	MAY 90	7	N	LIGHT
MJAV-9029		M	313.0	MAY 90	19	Y	MODER
MJAV-9030		F	204.0	MAY 90	0	N	NONE
MJAV-9031		M	295.0	JUL 90	5	N	NONE
MJAV-9032		M	125.0	JUL 90	0	N	NONE
MJAV-9033		M	340.0	JUL 90	14	N	LIGHT
MJAV-9034		M	295.0	JUL 90	13	N	MODER
MJAV-9035		F	180.0	JUL 90	0	N	NONE
MJAV-9036		M	290.0	AUG 90	7	N	LIGHT
MJAV-9037		M	320.0	AUG 90	11	N	LIGHT
MJAV-9038		F	148.0	SEP 90	0	N	NONE
MJAV-9039		F	150.0	SEP 90	0	N	NONE
MJAV-9040		M	164.0	SEP 90	0	N	NONE
MNE-9101		M	215.0	FEB 91	1	N	NONE
MNE-9103		F	215.0	MAR 91	1	N	NONE
MNE-9104		M	249.0	MAR 91	2	N	NONE
MNE-9105		F	305.0	APR 91	9	N	LIGHT
MNE-9106		M	147.0	APR 91	0	N	NONE
MNE-9107		M	135.0	APR 91	0	N	NONE
MNE-9109		M	148.0	MAY 91	0	N	NONE
MNE-9110		F	220.0	MAY 91	0	N	NONE
MNE-9111		F	120.0	MAY 91	0	N	NONE
MNE-9112		M	325.0	JUN 91	21	Y	MODER
MNE-9113		M	275.0	JUN 91	5	N	NONE
MNE-9114		M	327.0	JUN 91	30	Y	HEAVY
MNE-9115		F	148.0	JUL 91	0	N	NONE
MNE-9116		M	140.0	JUL 91	0	N	NONE
MNE-9119		F	300.0	AUG 91	9	N	LIGHT
MNE-9120		M	228.0	AUG 91	1	N	NONE
MNE-9121		M	230.0	AUG 91	1	N	NONE
MNE-9122		M	305.0	AUG 91	12	N	LIGHT
MNE-9124		F	157.0	AUG 91	0	N	NONE
MNE-9125		M	315.0	SEP 91	16	N	LIGHT

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
MNE-9126		U	240.0	AUG 91	6	N	LIGHT
MNE-9127		M	245.0	SEP 91	2	N	NONE
MNE-9130		M	328.0	OCT 91	24	Y	MODER
MNE-9131		M	348.0	OCT 91	20	N	MODER
MNE-9132		F	411.0	OCT 91	39	Y	HEAVY
MNE-9134		M	130.0	OCT 91	0	N	NONE
MNE-9136		M	138.0	NOV 91	0	N	NONE
MNW-8907		M	109.0	SEP 89	0	N	NONE
MNW-8908		M	290.0	OCT 89	9	N	LIGHT
MNW-8909		M	198.0	DEC 89	0	N	NONE
MNW-8910		F	200.0	DEC 89	1	N	NONE
MNW-9001		M	187.0	JAN 90	1	N	NONE
MNW-9002		F	330.0	JAN 90	16	N	MODER
MNW-9003		M	272.0	JAN 90	10	N	LIGHT
MNW-9004		F	234.0	JAN 90	1	N	NONE
MNW-9006		F	133.0	MAR 90	0	N	NONE
MNW-9007		F	189.0	MAR 90	4	N	NONE
MNW-9008		M	112.0	MAR 90	0	N	NONE
MNW-9013		F	147.0	JUL 90	0	N	NONE
MNW-9014		M	134.0	AUG 90	0	N	NONE
MNW-9016		M	320.0	SEP 90	16	N	MODER
MNW-9017		M	335.0	OCT 90	13	N	HEAVY
MNW-9018		M	283.0	OCT 90	8	N	LIGHT
MNW-9020		F	109.0	NOV 90	0	N	NONE
MNW-9021		U	310.0	NOV 90	10	N	MODER
MNW-9022		F	147.0	NOV 90	0	N	NONE
MNW-9023		M	319.0	NOV 90	13	Y	HEAVY
MNW-9024		F	300.0	NOV 90	9	N	NONE
MNW-9025		M	183.0	DEC 90	1	N	NONE
MNW-9101		F	140.0	JAN 91	0	N	NONE
MNW-9102		F	157.0	FEB 91	0	N	NONE
MNW-9103		M	271.0	FEB 91	4	N	NONE
MNW-9104		M	115.0	FEB 91	0	N	NONE
MNW-9105		F	129.0	MAR 91	0	N	NONE
MNW-9106		M	200.0	MAR 91	1	N	NONE
MNW-9107		M	123.0	MAR 91	0	N	NONE
MNW-9108		M	123.0	MAR 91	0	N	NONE
MNW-9110		M	127.0	MAR 91	0	N	NONE
MNW-9111		M	264.0	APR 91	6	N	NONE
MNW-9113		M	347.0	MAY 91	27	Y	HEAVY
MNW-9115		F	239.0	JUL 91	1	N	NONE
MNW-9116		F	242.0	JUL 91	3	N	NONE
MNW-9117		F	114.0	AUG 91	0	N	NONE
MNW-9118		M	239.0	AUG 91	1	N	NONE
MNW-9120		M	144.0	NOV 91	0	N	NONE
MSE-8801		F	230.0	MAR 88	1	N	NONE
MSE-8802		M	345.0	APR 88	26	N	MODER
MSE-8803		M	330.0	JUN 88	16	N	MODER
MSE-8901		F	228.0	JAN 89	1	N	NONE
MSE-8906		M	309.0	MAY 89	10	N	LIGHT
MSE-8908		F	318.0	MAY 89	10	N	MODER
MSE-8911		M	238.0	JUL 89	5	N	NONE
MSE-8914		M	130.0	AUG 89	0	N	NONE
MSE-8915		F	132.0	AUG 89	0	N	NONE
MSE-8916		M	122.0	SEP 89	0	N	NONE
MSE-8917		M	133.0	OCT 89	0	N	NONE
MSE-8919		F	208.0	DEC 89	0	N	NONE
MSE-9001		F	334.0	JAN 90	11	N	LIGHT
MSE-9003		M	332.0	JAN 90	25	Y	HEAVY
MSE-9004		M	185.0	JAN 90	0	N	NONE
MSE-9010		M	244.0	FEB 90	2	N	NONE
MSE-9011		F	275.0	APR 90	7	N	LIGHT
MSE-9012		F	295.0	APR 90	7	N	LIGHT
MSE-9018		M	314.0	AUG 90	14	N	MODER
MSE-9019		M	210.0	AUG 90	0	N	NONE
MSE-9021		M	285.0	DEC 90	8	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
MSE-9022		F	248.0	DEC 90	3	N	NONE
MSE-9023		F	308.0	NOV 90	6	N	LIGHT
MSE-9024		M	154.0	DEC 90	0	N	NONE
MSE-9101		M	335.0	JAN 91	37	Y	HEAVY
MSE-9102		F	272.0	JAN 91	5	N	LIGHT
MSE-9103		F	93.0	JAN 91	0	N	NONE
MSE-9105		M	323.0	FEB 91	27	Y	HEAVY
MSE-9106		M	263.0	APR 91	1	N	NONE
MSE-9107		M	232.0	APR 91	1	N	NONE
MSE-9108		U	282.0	MAY 91	8	N	LIGHT
MSE-9109		F	269.0	MAY 91	7	N	LIGHT
MSE-9110		F	285.0	JUN 91	5	N	NONE
MSE-9111		F	299.0	JUN 91	5	N	NONE
MSE-9112		M	313.0	JUN 91	15	N	LIGHT
MSE-9113		F	292.0	JUN 91	4	N	NONE
MSE-9114		F	166.0	JUL 91	0	N	NONE
MSE-9115		M	292.0	AUG 91	8	N	NONE
MSE-9116		M	223.0	AUG 91	2	N	NONE
MSE-9117		M	147.0	OCT 91	0	N	NONE
MSE-9118		M	320.0	OCT 91	13	N	MODER
MSE-9120		F	126.0	NOV 91	0	N	NONE
MSE-9125		M	200.0	DEC 91	0	N	NONE
MSW-018		M	294.5	MAR 84	10	N	LIGHT
MSW-019		F	246.0	MAR 84	2	N	NONE
MSW-020		M	203.0	MAR 84	0	N	NONE
MSW-025		M	290.0	MAY 84	7	N	LIGHT
MSW-026		F	147.0	JUN 84	0	N	NONE
MSW-029		M	210.0	JUN 84	1	N	NONE
MSW-030		F	138.0	AUG 84	0	N	NONE
MSW-032		M	162.0	SEP 84	0	N	NONE
MSW-034		F	232.0	OCT 84	2	N	NONE
MSW-035		M	272.0	OCT 84	6	N	NONE
MSW-036		M	205.0	OCT 84	1	N	NONE
MSW-037		F	376.0	NOV 84	16	N	LIGHT
MSW-038		M	239.0	DEC 84	1	N	NONE
MSW-039		M	210.0	JAN 85	1	N	NONE
MSW-040		F	194.0	JAN 85	1	N	NONE
MSW-041		M	233.0	JAN 85	1	N	NONE
MSW-042		M	208.0	FEB 85	1	N	NONE
MSW-044		M	112.0	MAR 85	0	N	NONE
MSW-045		F	115.0	MAR 85	0	N	NONE
MSW-047		M	177.0	MAR 85	0	N	NONE
MSW-048		M	212.0	MAR 85	1	N	NONE
MSW-049		M	204.0	APR 85	1	N	NONE
MSW-050		M	143.0	APR 85	0	N	NONE
MSW-051		F	166.0	APR 85	0	N	NONE
MSW-052		F	169.0	APR 85	0	N	NONE
MSW-053		F	216.5	MAY 85	1	N	NONE
MSW-054		M	133.0	MAY 85	0	N	NONE
MSW-056		M	280.0	JUN 85	9	N	LIGHT
MSW-058		M	150.0	JUN 85	0	N	NONE
MSW-059		U	120.0	JUN 85	0	N	NONE
MSW-063		M	281.0	AUG 85	5	N	NONE
MSW-066		M	123.0	SEP 85	0	N	NONE
MSW-067		M	235.0	NOV 85	1	N	NONE
MSW-070		F	235.0	JAN 86	1	N	NONE
MSW-071		M	266.0	JAN 86	1	N	NONE
MSW-072		M	190.0	JAN 86	1	N	NONE
MSW-073		F	231.0	FEB 86	1	N	NONE
MSW-074		M	184.0	FEB 86	0	N	NONE
MSW-075		F	318.0	MAR 86	15	N	MODER
MSW-076		F	196.0	MAR 86	1	N	NONE
MSW-077		M	110.0	MAR 86	0	N	NONE
MSW-078		M	278.0	MAR 86	7	N	NONE
MSW-079		F	259.0	MAR 86	7	N	NONE
MSW-080		F	255.0	MAR 86	2	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
MSW-081		F	295.0	MAR 86	9	N	LIGHT
MSW-082		M	256.0	APR 86	1	N	NONE
MSW-083		M	154.0	APR 86	0	N	NONE
MSW-084		F	251.0	APR 86	3	N	LIGHT
MSW-088		F	137.0	JUN 86	0	N	NONE
MSW-089		M	229.0	JUL 86	1	N	NONE
MSW-090		M	251.0	JUL 86	2	N	NONE
MSW-091		M	144.0	JUL 86	0	N	NONE
MSW-093		M	145.0	SEP 86	0	N	NONE
MSW-095		M	88.0	SEP 86	0	N	NONE
MSW-096		F	139.0	OCT 86	0	N	NONE
MSW-097		F	324.0	OCT 86	12	N	MODER
MSW-098		F	285.0	NOV 86	4	N	NONE
MSW-099		F	220.0	NOV 86	1	N	NONE
MSW-100		M	189.0	DEC 86	0	N	NONE
MSW-101		M	251.0	DEC 86	2	N	NONE
MSW-102		M	204.0	JAN 87	1	N	NONE
MSW-103		F	177.0	FEB 87	1	N	NONE
MSW-104		M	261.0	FEB 87	2	N	NONE
MSW-105		M	265.0	APR 87	5	N	NONE
MSW-106		F	308.0	APR 87	6	N	LIGHT
MSW-107		M	136.0	APR 87	0	N	NONE
MSW-109		F	300.0	MAY 87	7	N	NONE
MSW-111		F	330.0	JUN 87	13	N	MODER
MSW-112		F	251.0	JUN 87	3	N	NONE
MSW-113		M	244.0	JUL 87	2	N	NONE
MSW-114		M	327.0	JUL 87	24	Y	HEAVY
MSW-116		F	246.0	AUG 87	2	N	NONE
MSW-119		F	287.0	AUG 87	3	N	NONE
MSW-120		F	204.0	SEP 87	1	N	NONE
MSW-121		F	300.0	SEP 87	5	N	LIGHT
MSW-124		M	116.0	OCT 87	0	N	NONE
MSW-125		M	145.0	OCT 87	0	N	NONE
MSW-126		M	253.0	NOV 87	1	N	NONE
MSW-127		F	325.0	NOV 87	15	N	MODER
MSW-128		M	287.0	DEC 87	17	N	MODER
MSW-129		M	134.0	DEC 87	0	N	NONE
MSW-130		M	190.0	DEC 87	1	N	NONE
MSW-131		F	237.0	DEC 87	1	N	NONE
MSW-132		M	70.0	DEC 87	0	N	NONE
MSW-133		F	302.0	DEC 87	10	N	MODER
MSW-138		M	242.0	FEB 88	5	N	NONE
MSW-139		M	209.0	MAR 88	1	N	NONE
MSW-140		M	203.0	MAR 88	1	N	NONE
MSW-141		M	195.0	MAR 88	2	N	NONE
MSW-142		F	240.0	MAR 88	3	N	NONE
MSW-143		M	314.0	MAR 88	18	N	MODER
MSW-145		M	277.0	APR 88	4	N	NONE
MSW-146		M	170.0	APR 88	0	N	NONE
MSW-149		M	149.0	MAY 88	0	N	NONE
MSW-150		M	140.0	MAY 88	0	N	NONE
MSW-151		F	324.0	MAY 88	12	N	MODER
MSW-153		M	244.0	MAY 88	0	N	NONE
MSW-155		F	132.0	JUN 88	0	N	NONE
MSW-156		M	114.0	JUN 88	0	N	NONE
MSW-159		M	134.0	JUN 88	0	N	NONE
MSW-160		M	99.0	JUN 88	0	N	NONE
MSW-162		F	281.0	JUN 88	7	N	NONE
MSW-166		M	329.0	JUL 88	11	N	MODER
MSW-169		M	193.0	JUL 88	0	N	NONE
MSW-171		M	345.0	AUG 88	19	N	MODER
MSW-172		F	309.0	AUG 88	7	N	LIGHT
MSW-174		F	296.0	AUG 88	7	N	LIGHT
MSW-176		F	325.0	SEP 88	12	N	LIGHT
MSW-179		M	241.0	OCT 88	3	N	NONE
MSW-180		F	371.0	NOV 88	36	Y	HEAVY

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
MSW-181		F	171.0	DEC 88	1	N	NONE
MSW-182		M	210.0	DEC 88	3	N	NONE
MSW-183		M	166.0	DEC 88	0	N	NONE
MSW-184		F	302.0	DEC 88	5	N	NONE
MSW-185		F	224.0	DEC 88	1	N	NONE
MSW-188		M	298.0	JAN 89	12	N	LIGHT
MSW-190		M	223.0	JAN 89	2	N	NONE
MSW-191		M	239.0	JAN 89	2	N	NONE
MSW-202		F	202.0	APR 89	1	N	NONE
MSW-205		M	208.0	APR 89	0	N	NONE
MSW-208		M	237.0	APR 89	2	N	NONE
MSW-212		M	182.0	MAY 89	1	N	NONE
MSW-213		F	287.0	JUN 89	6	N	LIGHT
MSW-214		F	347.0	JUN 89	26	Y	HEAVY
MSW-216		M	315.0	JUL 89	25	Y	HEAVY
MSW-217		F	370.0	JUL 89	13	N	MODER
MSW-219		M	212.0	AUG 89	1	N	NONE
MSW-223		F	252.0	SEP 89	2	N	NONE
MSW-224		M	256.0	SEP 89	2	N	NONE
MSW-225		F	209.0	SEP 89	2	N	NONE
MSW-226		M	140.0	SEP 89	0	N	NONE
MSW-229		F	231.0	OCT 89	2	N	NONE
MSW-230		F	245.0	NOV 89	3	N	NONE
MSW-231		M	235.0	NOV 89	2	N	NONE
MSW-232		M	194.0	NOV 89	1	N	NONE
MSW-233		F	199.0	DEC 89	0	N	NONE
MSW-234		M	197.0	DEC 89	0	N	NONE
MSW-235		F	253.0	DEC 89	4	N	NONE
MSW-236		M	200.0	DEC 89	1	N	NONE
MSW-237		M	333.0	DEC 89	28	Y	HEAVY
MSW-238		M	211.0	DEC 89	1	N	NONE
MSW-239		F	228.0	JAN 90	1	N	NONE
MSW-240		F	370.0	JAN 90	20	Y	HEAVY
MSW-241		F	307.0	JAN 90	16	N	MODER
MSW-242		M	191.0	JAN 90	1	N	NONE
MSW-243		F	191.0	JAN 90	1	N	NONE
MSW-244		F	167.0	JAN 90	0	N	NONE
MSW-245		M	143.0	JAN 90	0	N	NONE
MSW-246		F	292.0	JAN 90	8	N	LIGHT
MSW-247		M	225.0	JAN 90	1	N	NONE
MSW-248		M	219.0	JAN 90	1	N	NONE
MSW-251		M	230.0	JAN 90	3	N	NONE
MSW-253		F	228.0	JAN 90	1	N	NONE
MSW-254		F	197.0	FEB 90	0	N	NONE
MSW-255		U	253.0	FEB 90	2	N	NONE
MSW-257		M	366.0	FEB 90	13	N	MODER
MSW-258		F	175.0	FEB 90	0	N	NONE
MSW-259		M	221.0	FEB 90	2	N	NONE
MSW-260		F	147.0	MAR 90	0	N	NONE
MSW-261		M	140.0	MAR 90	0	N	NONE
MSW-262		M	288.0	MAR 90	6	N	NONE
MSW-263		M	125.0	MAR 90	0	N	NONE
MSW-264		F	304.0	APR 90	21	N	HEAVY
MSW-266		U	300.0	APR 90	8	N	LIGHT
MSW-268		F	256.0	APR 90	2	N	NONE
MSW-271		F	274.0	MAY 90	3	N	NONE
MSW-272		M	295.0	MAY 90	6	N	NONE
MSW-273		M	139.0	MAY 90	0	N	NONE
MSW-275		F	273.0	JUL 90	7	N	LIGHT
MSW-276		F	138.0	JUL 90	0	N	NONE
MSW-277		F	337.0	JUL 90	19	Y	HEAVY
MSW-278		M	238.0	JUL 90	1	N	NONE
MSW-279		F	344.0	AUG 90	19	N	HEAVY
MSW-281		F	252.0	AUG 90	6	N	NONE
MSW-282		F	140.0	SEP 90	0	N	NONE
MSW-283		M	147.0	SEP 90	0	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
MSW-284		F	123.0	NOV 90	0	N	NONE
MSW-285		F	197.0	NOV 90	0	N	NONE
MSW-286		M	251.0	DEC 90	2	N	NONE
MSW-287		M	302.0	DEC 90	17	N	HEAVY
MSW-288		F	315.0	DEC 90	12	N	HEAVY
MSW-290		F	313.0	DEC 90	26	Y	HEAVY
MSW-291		M	282.0	DEC 90	10	N	LIGHT
MSW-292		M	191.0	DEC 90	0	N	NONE
MSW-293		F	192.0	DEC 90	0	N	NONE
MSW-9101		M	325.0	JAN 91	26	Y	HEAVY
MSW-9102		M	217.0	JAN 91	1	N	NONE
MSW-9103		F	365.0	MAR 91	18	Y	HEAVY
MSW-9104		M	222.0	MAR 91	0	N	NONE
MSW-9106		M	144.0	MAR 91	0	N	NONE
MSW-9107		M	338.0	APR 91	27	N	HEAVY
MSW-9108		U	120.0	APR 91	0	N	NONE
MSW-9109		F	260.0	APR 91	6	N	NONE
MSW-9110		F	235.0	APR 91	2	N	NONE
MSW-9111		F	305.0	APR 91	7	N	LIGHT
MSW-9112		F	285.0	APR 91	7	N	NONE
MSW-9113		M	293.0	MAY 91	8	N	LIGHT
MSW-9114		F	340.0	MAY 91	34	Y	HEAVY
MSW-9115		F	360.0	MAY 91	35	Y	HEAVY
MSW-9116		M	210.0	JUN 91	1	N	NONE
MSW-9117		F	284.0	JUN 91	7	N	NONE
MSW-9120		F	350.0	JUL 91	29	Y	HEAVY
MSW-9122		M	123.0	JUL 91	0	N	NONE
MSW-9123		F	336.0	AUG 91	12	N	MODER
MSW-9124		F	295.0	AUG 91	7	N	NONE
MSW-9125		F	131.0	AUG 91	0	N	NONE
MSW-9127		F	119.0	AUG 91	0	N	NONE
MSW-9128		F	128.0	AUG 91	0	N	NONE
MSW-9129		M	104.0	AUG 91	0	N	NONE
MSW-9130		M	228.0	SEP 91	1	N	NONE
MSW-9131		M	292.0	SEP 91	13	N	MODER
MSW-9132		F	251.0	SEP 91	1	N	NONE
MSW-9133		M	159.0	OCT 91	0	N	NONE
MSW-9134		F	308.0	OCT 91	12	N	MODER
MSW-9135		F	249.0	OCT 91	1	N	NONE
MSW-9137		M	289.0	OCT 91	14	N	LIGHT
MSW-9138		F	122.0	NOV 91	0	N	NONE
MSW-9140		F	270.0	NOV 91	2	N	NONE
MSW-9141		M	252.0	NOV 91	5	N	NONE
MSW-9142		F	229.0	NOV 91	2	N	NONE
MSW-9144		F	286.0	DEC 91	7	N	NONE
MSW-9145		F	209.0	DEC 91	0	N	NONE
SWF-8509		F	250.0	SEP 85	2	N	NONE
SWF-8512		M	205.0	SEP 85	0	N	NONE
SWF-8518		F	285.0	NOV 85	5	N	NONE
SWF-8519		F	225.0	NOV 85	1	N	NONE
SWF-8521		F	210.0	DEC 85	1	N	NONE
SWF-8617		M	133.0	APR 86	0	N	NONE
SWF-8621		M	121.0	MAY 86	0	N	NONE
SWF-8623		M	246.0	MAY 86	1	N	NONE
SWF-8624		F	217.0	MAY 86	0	N	NONE
SWF-8625		M	140.0	MAY 86	0	N	NONE
SWF-8629		F	132.0	JUN 86	0	N	NONE
SWF-8630		F	246.0	JUN 86	3	N	NONE
SWF-8631		M	283.0	JUN 86	5	N	NONE
SWF-8632		M	244.0	JUL 86	2	N	NONE
SWF-8639		F	165.0	JUL 86	0	N	NONE
SWF-8643		F	134.0	JUL 86	0	N	NONE
SWF-8659		M	105.0	NOV 86	0	N	NONE
SWF-8701		M	146.0	JAN 87	0	N	NONE
SWF-89128		M	127.0	AUG 89	0	N	NONE
SWF-89158		F	106.0	OCT 89	0	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
SWF-8919		M	267.0	NOV 89	9	N	LIGHT
SWF-9007		F	121.0	MAR 90	0	N	NONE
SWF-9008		F	302.0	APR 90	3	N	NONE
SWF-9011		F	260.0	APR 90	3	N	NONE
UCF-8901		M	125.0	NOV 89	0	N	NONE
UCF-8903		M	281.0	DEC 89	8	N	LIGHT
UCF-9001		F	170.0	DEC 89	0	N	NONE
UCF-9002		M	160.0	JAN 90	0	N	NONE
UCF-9003		F	290.0	JAN 90	8	N	LIGHT
UCF-9005		F	140.0	JAN 90	0	N	NONE
UCF-9006		M	240.0	JAN 90	3	N	NONE
UCF-9007		F	250.0	JAN 90	0	N	NONE
UCF-9009		M	148.0	JAN 90	0	N	NONE
UCF-9010		F	329.0	JAN 90	11	N	LIGHT
UCF-9011		M	221.0	JAN 90	1	N	NONE
UCF-9012		F	267.0	JAN 90	3	N	NONE
UCF-9013		F	209.0	JAN 90	1	N	NONE
UCF-9014		F	254.0	JAN 90	3	N	NONE
UCF-9015		M	240.0	JAN 90	2	N	NONE
UCF-9016		F	272.0	JAN 90	5	N	NONE
UCF-9017		F	235.0	JAN 90	2	N	NONE
UCF-9018		M	208.0	JAN 90	1	N	NONE
UCF-9019		M	268.0	JAN 90	5	N	NONE
UCF-9020		M	190.0	JAN 90	0	N	NONE
UCF-9021		F	284.0	JAN 90	4	N	NONE
UCF-9022		F	262.0	JAN 90	2	N	NONE
UCF-9023		F	168.0	JAN 90	0	N	NONE
UCF-9024		M	243.0	JAN 90	1	N	NONE
UCF-9025		F	187.0	JAN 90	0	N	NONE
UCF-9026		F	253.0	JAN 90	1	N	NONE
UCF-9027		M	232.0	JAN 90	1	N	NONE
UCF-9028		M	275.0	JAN 90	6	N	NONE
UCF-9029		M	166.0	JAN 90	0	N	NONE
UCF-9030		M	198.0	JAN 90	0	N	NONE
UCF-9032		F	214.0	JAN 90	1	N	NONE
UCF-9033		M	229.0	JAN 90	1	N	NONE
UCF-9034		F	268.0	FEB 90	3	N	NONE
UCF-9035		F	262.0	MAR 90	5	N	NONE
UCF-9036		U	282.0	MAR 90	4	N	NONE
UCF-9038		F	116.0	MAR 90	0	N	NONE
UCF-9039		F	99.0	MAR 90	0	N	NONE
UCF-9040		F	119.0	MAR 90	0	N	NONE
UCF-9041		F	257.0	MAR 90	3	N	NONE
UCF-9042		F	310.0	MAR 90	10	N	LIGHT
UCF-9043		F	280.0	MAR 90	6	N	NONE
UCF-9044		M	235.0	MAR 90	2	N	NONE
UCF-9045		F	323.0	MAR 90	8	N	NONE
UCF-9046		M	240.0	OCT 90	2	N	NONE
UCF-9048		M	113.0	APR 90	0	N	NONE
UCF-9050		M	115.0	JUN 90	0	N	NONE
UCF-9051		M	113.0	JUN 90	0	N	NONE
UCF-9053		F	307.0	AUG 90	6	N	NONE
UCF-9054		F	250.0	AUG 90	2	N	NONE
UCF-9056		F	177.0	AUG 90	0	N	NONE
UCF-9057		F	168.0	SEP 90	0	N	NONE
UCF-9058		F	290.0	OCT 90	4	N	NONE
UCF-9059		F	308.0	OCT 90	7	N	LIGHT
UCF-9060		M	139.0	NOV 90	0	N	NONE
UCF-9061		M	130.0	DEC 90	0	N	NONE
UCF-9101		M	154.0	JAN 91	0	N	NONE
UCF-9102		M	195.0	FEB 91	0	N	NONE
UCF-9103		M	209.0	FEB 91	1	N	NONE
UCF-9104		M	159.0	FEB 91	0	N	NONE
UCF-9105		M	285.0	FEB 91	9	N	NONE
UCF-9106		F	135.0	FEB 91	0	N	NONE
UCF-9107		F	335.0	MAR 91	25	Y	HEAVY

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
UCF-9108		F	128.0	MAR 91	0	N	NONE
UCF-9109		U	84.0	MAR 91	0	N	NONE
UCF-9110		U	180.0	MAR 91	0	N	NONE
UCF-9111		F	206.0	MAR 91	1	N	NONE
UCF-9112		F	325.0	APR 91	17	Y	HEAVY
UCF-9113		F	232.0	APR 91	1	N	NONE
UCF-9114		F	293.0	APR 91	6	N	LIGHT
UCF-9116		U	225.0	APR 91	5	N	NONE
UCF-9118		M	221.0	MAY 91	2	N	NONE
UCF-9119		F	260.0	MAY 91	3	N	NONE
UCF-9120		M	310.0	JUN 91	14	N	LIGHT
UCF-9122		F	213.0	JUN 91	1	N	NONE
UCF-9123		M	203.0	JUN 91	0	N	NONE
UCF-9124		M	105.0	JUN 91	0	N	NONE
UCF-9125		M	149.0	JUN 91	0	N	NONE
UCF-9127		M	105.0	JUL 91	0	N	NONE
UCF-9129		M	275.0	AUG 91	7	N	LIGHT
UCF-9130		M	125.0	AUG 91	0	N	NONE
UCF-9133		F	380.0	SEP 91	25	Y	HEAVY
UCF-9135		F	127.0	OCT 91	0	N	NONE
UCF-9136		M	330.0	OCT 91	22	Y	HEAVY
UCF-9137		M	220.0	OCT 91	1	N	NONE
UCF-9138		F	296.0	OCT 91	6	N	NONE
UCF-9141		M	306.0	DEC 91	8	N	NONE
KDLUK8627		U	0.0		0	N	NONE
KDLUK8643		U	0.0		0	N	NONE
KDLUK8729		U	0.0		0	N	NONE
KDLUK8731		U	0.0		1	N	NONE
KDLUK8748		U	0.0		0	N	NONE
KDLUK8757B		U	0.0		0	N	NONE
KDLUK87__A		U	0.0		1	N	NONE
KDLUK87__B		U	0.0		0	N	NONE
KDLUK8804		U	0.0		12	N	LIGHT
KDLUK8907		U	0.0		0	N	NONE
KDLUK8915		U	0.0		1	N	NONE
MUK151		U	0.0		13	N	MODER
MUK7610		U	0.0		5	N	NONE
MUK7726		U	0.0		1	N	NONE
MUK7801		U	0.0		3	N	NONE
MUK7803		U	0.0		20	N	MODER
MUK7911		U	0.0		6	N	NONE
MUK8163		U	0.0		5	N	LIGHT
MUK8217		U	0.0		18	N	HEAVY
MUK8312		U	0.0		1	N	NONE
MUK8609		U	0.0		8	N	LIGHT
MUK8612		U	0.0		3	N	NONE
MUK8		U	0.0		27	Y	HEAVY
MUKODELL		U	0.0		3	N	NONE
MUKSKULL		U	0.0		4	N	NONE
MJAVUK8907		U	0.0		13	N	LIGHT
MJAVUK9009		U	0.0		10	N	LIGHT
MJAVUK9023		U	0.0		3	N	NONE
MSELUK8804		U	0.0		5	N	NONE
MSELUK9120		U	0.0		10	N	LIGHT
MSQUK8177		U	0.0		0	N	NONE
MSWUK009		U	0.0		22	Y	MODER
MSWUK068		U	0.0		1	N	NONE
MSWUK185		U	0.0		0	N	NONE
MSWUK448		U	0.0		1	N	NONE
MSWUK9120		U	0.0		29	Y	HEAVY
MSWUKA		U	0.0		0	N	NONE
MSWUKB		U	0.0		1	N	NONE
MSWUKC		U	0.0		4	N	NONE
MSWUKD		U	0.0		13	N	MODER
MSWUKE		U	0.0		3	N	NONE
MSWUKF		U	0.0		0	N	NONE

Museum Number	Salvage Number	Sex	Length (cm)	Death Date	Age	Estimated Age?	Resorption
	SWFUK8509	U	0.0		0	N	NONE
	UCFUK9010	U	0.0		3	N	NONE
	UCFUK?	U	0.0		0	N	NONE
	UK#4"	U	0.0		0	N	NONE
	UK#5"	U	0.0		3	N	NONE
	UK03	U	0.0		10	N	NONE
	UK06, 90	U	0.0		4	N	LIGHT
	UK07	U	0.0		1	N	NONE
	UK10	U	0.0		4	N	NONE
	UK160R91	U	0.0		1	N	LIGHT
	UK33	U	0.0		0	N	NONE
	UK42	U	0.0		0	N	NONE
	UKKF08*	U	0.0		3	N	NONE
	UKKF1033	U	0.0		2	N	NONE
	UKKF1034	U	0.0		3	N	NONE
	UKKF1035	U	0.0		9	N	NONE
	UKKF1036	U	0.0		0	N	LIGHT
	UKKF1037	U	0.0		3	N	NONE
	UKKF1038	U	0.0		6	N	NONE
	UKKF1043B	U	0.0		8	N	LIGHT
	UKNBA	U	0.0		1	N	LIGHT
	UKNBB	U	0.0		6	N	NONE
	UKNM530314	U	0.0		0	N	NONE
	UKPALATEHOL	U	0.0		5	N	NONE
	UKTRASH	U	0.0		21	N	LIGHT
	UKZYG	U	0.0		6	N	MODER LIGHT

APPENDIX B

MUSEUM CATALOG NUMBERS, SALVAGE NUMBERS, FIELD OR OCEANARIA NUMBERS, AND NAMES OF INDIVIDUAL MANATEES USED IN THIS STUDY WHERE MORE THAN ONE IDENTIFICATION NUMBER OR NAME HAS BEEN ASSIGNED

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
CAS 23278	M-260		
CAS 23279	M-84-22		
CAS 23280	M-273		
CAS 23281	M-82-20		
CAS 23282	M-82-14		
CAS 23283	M-81-15		
CAS 23285	M-268		
CAS 23286	M-334		
CAS 23287	M-81-67		
CAS 23288	M-83-20		
CAS 23289	M-81-66		
CAS 23290	M-82-03		
CAS 23291	M-82-56		
CAS 23293	M-82-52		
CIMNH 733	M-364		
CIMNH 734	M-366		
CIMNH 735	M-367		
CIMNH 736	M-368		
CIMNH 737	M-370		
CIMNH 738	M-372		
CIMNH 740	M-82-30		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
CIMNH 741	M-82-31		
CIMNH 742	M-82-34		
CIMNH 743	M-82-39		
CIMNH 744	M-82-40		
CIMNH 745	M-82-43		
CIMNH 746	M-82-44		
CIMNH 748	M-82-50		
CIMNH 749	M-82-53		
CIMNH 750	M-82-55		
CIMNH 751	M-82-57		
CM 77798	M-219		
CM 77800	M-224		
CM 77802	M-199		
CM 77803	M-194		
CM 77804	M-234		
CM 77805	M-197		
CM 77806	M-201		
CM 77807	M-189		
CM 77808	M-230		
CM 77809	M-240		
CM 77810	M-237		
CM 77811	M-223		
CM 77812	M-226		
CM 77813	M-196		
CM 77814	M-212		
CM 77815	M-222		
CM 77816	M-209		
CM 77819	M-235		
LACM 72225	M-218		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
LACM 72226	M-225		
LACM 72227	M-229		
LACM 72228	M-239		
LACM 72229	M-244		
LACM 72230	M-246		
LACM 72231	M-249		
LACM 72232	M-250		
LACM 72233	M-254		
LACM 72234	M-255		
LACM 72235	M-256		
LACM 72236	M-259		
LACM 72238	M-262		
LACM 72239	M-266		
LACM 72240	M-275		
LACM 72244	M-292		
LACM 72246	M-328		
LACM 72249	M-343		
LACM 72250	M-361		
LACM 72252	M-81-16		
LACM 72254	M-81-26		
LACM 72256	M-81-59		
LACM 72258	M-81-68		
LACM 72259	M-82-02		
LACM 72260	M-82-07		
LACM 72261	M-82-10		
LACM 72262	M-82-15		
LACM 72263	M-82-18		
LACM 72266	M-82-24		
LACM 72267	M-82-25		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
LACM 72268	M-82-29		
LACM 72270	M-82-33		
LACM 72272	M-82-36		
MSB 50450	M-269		
MSB 50454	M-81-08		
MSB 50455	M-81-62		
MSB 50456	M-81-61		
MSB 50457	M-81-49		
MSB 50460	M-81-36		
MSB 50461	M-81-05		
MSB 50463	M-274		
MSB 50464	M-81-38		
MSB 50465	M-81-35		
MSB 50466	M-81-60?		
MSB 50467	M-81-34		
MSB 50468	M-81-24		
MSB 50469	M-81-44		
NCSM 3618	M-019	UF 13575	
NCSM 4563	M-388		
NCSM 4564	M-247		
NCSM 4565	M-338		
NCSM 4566	M-355		
NCSM 4567	M-339		
NCSM 4568	M-326		
NCSM 4570	M-342		
NCSM 4571	M-347		
NCSM 4572	M-310		
NCSM 4573	M-337		
NCSM 4574	M-358		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
NCSM 4575	M-340		
NCSM 4576	M-360		
NCSM 4577	M-348		
NCSM 5252	M-462		
NCSM 5426	MSW-014		
NCSM 5429	M-280		
NCSM 5430	M-288		
NCSM 5431	M-322		
NCSM 5432	M-333		
NCSM 5433	M-369		
NCSM 5434	M-373		
NCSM 5435	M-81-71		
NCSM 5436	M-82-01		
NCSM 5437	M-82-51		
NCSM 5438	M-83-02		
NCSM 5439	M-83-05		
NCSM 5440	M-83-10		
NCSM 5441	M-83-13		
NCSM 5442	M-83-14		
NCSM 5443	M-83-17		
NCSM 5444	M-83-23		
NCSM 5445	M-83-34		
NCSM 5446	M-83-37		
NCSM 5447	M-83-40		
NCSM 5448	M-84-06		
NCSM 5449	M-84-18		
NCSM 5450	M-84-27		
NCSM 6658	M-83-24		
NCSM 6659	M-84-13		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
NM 527899	M-094		
NM 527900	M-095		
NM 527901	M-096		
NM 527903	M-098		
NM 527905	M-100		
NM 527911	M-107		
NM 527912	M-108		
NM 527913	M-109		
NM 527916	M-112		
NM 527918	M-115		
NM 527920	M-117		
NM 527921	M-118		
NM 527922	M-119		
NM 527923	M-120		
NM 527925	M-122		
NM 527926	M-123		
NM 527927	M-124		
NM 530294	M-128		
NM 530297	M-131		
NM 530298	M-132		
NM 530299	M-155		
NM 530300	M-75-05		
NM 530301	M-77-07		
NM 530302	M-77-09		
NM 530303	M-77-16		
NM 530304	M-77-20		
NM 530305	M-77-21		
NM 530306	M-77-22		
NM 530310	M-78-02		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
NM 530311	M-78-08		
NM 530312	M-78-09		
NM 530313	M-78-10		
NM 530314	M-78-11		
NM 530314	M-78-15		
NM 530316	M-78-16		
NM 530318	M-78-20		
NM 530320	M-78-33		
NM 530322	M-78-39		
NM 530324	M-78-42		
NM 530325	M-78-44		
NM 530327	M-79-05		
NM 530328	M-79-06		
NM 550321	M-198		
NM 550329	M-276		
NM 550416	M-203		
NM 550417	M-281		
NM 550419	M-283		
NM 550421	M-331		
NM 551656	M-133		
NM 551657	M-134		
NM 551658	M-135		
NM 551660	M-137		
NM 551663	M-140		
NM 551666	M-144		
NM 551668	M-148		
NM 551670	M-154		
NM 551671	M-156		
NM 551672	M-146		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
NM 551673	M-160		
NM 551674	M-162		
NM 551675	M-167		
NM 551676	M-172		
NM 551677	M-173		
NM 551679	M-177		
NM 551680	M-178		
NM 551681	M-143		
NM 554180	M-179		
OMNH 17189	M-296		
OMNH 17190	M-302		
OMNH 17192	M-313		
OMNH 17194	M-321		
OMNH 17195	M-323		
OMNH 17196	M-324		
OMNH 17198	M-330		
OMNH 17201	M-82-08		
OMNH 17202	M-82-11		
OMNH 17203	M-82-12		
OMNH 17204	M-82-19		
OMNH 17205	M-82-21		
OSU 11710	M-279		
OSU 11711	M-350		
OSU 11713	M-83-15		
OSU 11714	M-82-16		
OSU 11716	M-392		
OSU 11717	M-332		
OSU 11718	M-216		
OSU 11720	M-83-08		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
OSU 11721	M-263		
OSU 11722	M-264		
OSU 11723	M-217		
OSU 11724	M-294		
OSU 11726	M-384		
OSU 11727	M-81-65		
OSU 11728	M-82-09		
OSU 11729	M-81-63		
UF 13553	M-015		
UF 13571	M-018		
UF 13877	M-036		
UF 13889	M-040		
UF 13892	M-043		
UF 13894	M-045		
UF 13900	M-049		
UF 13920	M-069		
UF 13923	M-072		
UF 13988	M-077		
UF 13991	M-078		
UF 14207	M-091		
UF 14815	M-083		
UF 15110	M-147		
UF 15111	M-149		
UF 15112	M-150		
UF 15113	M-151		
UF 15114	M-153		
UF 15115	M-157		
UF 15116	M-158		
UF 15118	M-161		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
UF 15119	M-163		Gilmore
UF 15120	M-164		
UF 15121	M-165		
UF 15122	M-166		Pope
UF 15123	M-168		
UF 15125	M-170		
UF 15126	M-171		
UF 15127	M-174		
UF 15128	M-175		
UF 15129	M-180		
UF 15130	M-182		
UF 15132	M-188		
UF 15133	M-190		
UF 15134	M-191		
UF 15135	M-193		
UF 15136	M-213		
UF 15137	M-214		
UF 15138	M-221		
UF 15139	M-228		
UF 15140	M-231		
UF 15141	M-232		
UF 15142	M-233		
UF 15143	M-238		
UF 15144	M-241		
UF 15145	M-243		
UF 15146	M-245		
UF 15150	M-75-08		
UF 15151	M-80-14		
UF 15142	M-75-10		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
UF 15157	M-77-39		
UF 15159	M-78-06		
UF 15160	M-78-07		
UF 15161	M-78-12		
UF 15162	M-78-22		
UF 15163	M-78-37		
UF 15165	M-79-01		
UF 15169	M-79-08		
UF 15170	M-79-09		Henry
UF 15171	M-79-17		
UF 15172	M-79-18		
UF 15173	M-79-20		
UF 15174	M-79-21		
UF 15175	M-79-22		
UF 15176	M-79-24		
UF 15177	M-79-25		
UF 15178	M-79-26		
UF 15179	M-80-01		
UF 15180	M-80-02		
UF 15181	M-80-03		
UF 15182	M-80-04		
UF 15184	M-80-06		
UF 15185	M-80-07		
UF 15186	M-80-09		
UF 15187	M-80-10		
UF 15188	M-80-11		
UF 15189	M-80-12		
UF 15190	M-80-13		
UF 15192	M-80-15		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
UF 15193	M-80-16		
UF 15196	M-80-18		
UF 15197	M-80-19		
UF 15198	M-80-20		
UF 15199	M-80-21		
UF 15200	M-80-22		
UF 15202	M-80-24		
UF 15203	M-80-25		
UF 15204	M-80-26		
UF 15206	M-81-01		
UF 15207	M-81-03		
UF 15208	M-81-04		
UF 15209	M-81-10		
UF 15210	M-81-43		
UF 15211	M-81-48		
UF 15215	M-81-53		
UF 15216	M-81-54		
UF 15217	M-81-55		
UF 19134	M-81-56		
UF 19135	M-81-57		
UF 20592	M-379		
UF 20594	M-412		
UF 20595	M-413		
UF 20596	M-414		
UF 20597	M-415		
UF 20598	M-423		
UF 20599	M-428		
UF 20600	M-432		
UF 20601	M-435		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
UF 20602	M-444		
UF 20603	MSW-001		
UF 20604	MSW-009		
UF 20605	MSW-021		
UF 20606	MSW-024		
UF 20607	M-83-36		
UF 20608	M-84-14		Nurdine
UF 20609	M-84-30		
UF 20757	M-202	SWFTm-927B	Panama
UF 20758	M-311	MSQ-6	Alexandra
UF 20773	M-316	BS-6	Gandalf
UF 20774	M-83-01	SWFTm-8021B	Violet
UF 22380	M-84-33		
UF 23993	M-386	BS-47	Walter
UF 24950	M-181		Laura
UF 24953	M-270		
UF 24954	M-299		
UF 24955	M-300		
UF 24956	M-307		
UF 24958	M-318		
UF 24959	M-336		
UF 24960	M-346		
UF 24961	M-349		
UF 24962	M-352		
UF 24963	M-353		
UF 24964	M-356		
UF 24965	M-365		
UF 24966	M-371		
UF 24967	M-374		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
UF 24968	M-375		
UF 24969	M-376		
UF 24970	M-381		
UF 24971	M-382	CR-39	Josephine
UF 24972	M-389		
UF 24975	M-391		
UF 24977	M-396		
UF 24978	M-398		
UF 24980	M-401		
UF 24985	M-410		
UF 24988	M-417		
UF 24989	M-422		Dihedral
UF 24990	M-425		
UF 24991	M-426		
UF 24993	M-439		
UF 24996	M-445		
UF 24998	M-448		
UF 25000	M-450		
UF 25001	M-452		
UF 25003	M-456		
UF 25005	M-457		
UF 25006	M-458		
UF 25010	M-74-04		
UF 25011	M-75-01		
UF 25012	M-75-13		
UF 25013	M-75-14		
UF 25014	M-76-05		
UF 25015	M-76-07		
UF 25017	M-76-16		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
UF 25018	M-76-19		
UF 25019	M-76-21		
UF 25020	M-76-23		
UF 25021	M-76-29		
UF 25022	M-77-14		
UF 25023	M-77-15		
UF 25024	M-77-31		
UF 25025	M-77-33		
UF 25026	M-77-34		
UF 25027	M-77-35		
UF 25028	M-77-36		
UF 25029	M-77-38		
UF 25030	M-77-41		
UF 25031	M-77-42		
UF 25032	M-78-13		
UF 25033	M-78-14		
UF 25034	M-78-27		
UF 25035	M-78-32		
UF 25036	M-78-36		
UF 25037	M-79-10		
UF 25038	M-79-23		
UF 25039	M-82-05		
UF 25040	M-82-06		
UF 25041	M-82-17		
UF 25042	M-82-26		
UF 25044	M-82-45		
UF 25045	M-82-47		
UF 25046	M-82-48		
UF 25047	M-83-03		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
UF 25048	M-83-04		
UF 25049	M-83-06		
UF 25050	M-83-07		
UF 25051	M-83-09		
UF 25054	M-83-27		
UF 25055	M-83-28		
UF 25058	M-83-32		
UF 25059	M-83-33		
UF 25061	M-83-38		
UF 25062	M-83-39		
UF 25063	M-84-01		
UF 25064	M-84-02		
UF 25066	M-84-05		
UF 25067	M-84-15		
UF 25068	M-84-16		
UF 25069	M-84-19		
UF 25071	M-84-25		
UF 25072	M-84-26		
UF 25073	M-84-28		
UF 25074	M-84-31		
UF 25075	M-84-32		
UF 25076	M-85-02		
UF 25077	M-85-03		
UF 25078	M-85-05		
UF 25079	M-85-06		
UF 25080	M-85-07		
UF 25081	M-85-10		
UF 25082	M-85-11		
UWBM 34504	MSW-015		

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
UWBM 34506	M-83-16		
UWBM 34507	M-84-07		
UWBM 34508	M-84-08		
UWBM 34509	M-84-11		
UWBM 34510	M-84-12		
UWBM 34511	M-84-17		
UWBM 34512	M-84-23		
UWBM 34514	M-208		
UWBM 34515	M-286		
UWBM 34517	M-354		
UWBM 34518	M-362		
UWBM 34519	M-377		
UWBM 34520	M-383		
UWBM 34521	M-397		
UWBM 34522	M-395		
	KDL-8704	KF-116	
	KDL-8744		Angela
	KDL-8821		Slash
	KDL-8827	BS-36	Felicity
	KDL-8839		Choptail
	M-453	BC-69	
	MJAV-8921		Lester
	MJAV-9026		Mary
	MNE-9102	BS-17	Sweetgums
	MNE-9131		Pincer
	MNW-8907		Corky
	SWFTm-7918B		Marina
	SWFTm-8321B		Lefty
	SWFTm-8646B	BS-9	Emma

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
	SWFTm-8745		Spock
	SWFTm-8752		Frederick
	SWFTm-8869B		Fogey
	SWFTm-8904B		Slim
	SWFTm-8908		Ranger
	SWFTm-8912B		Moss/Ross
	SWFTm-8917B		Susie
	SWFTm-9002B	BS-87	Liza
	SWFTm-9007		Disney
	SWFTm-9105		Mary
	SWFTm-9215B	BS-05	Phoebe
	UCF-8903	or 9003	
	UCF-8905		Leroy?
	UCF-9059		Ruth, Abheer
	UCF-9131		Ernie
	KDLUK87_A	(30-50)	
	KDLUK87_B	KF-57	
	MSWUKA	KF-955	
	MSWUKB	KF-956	
	MSWUKC	KF-957	
	MSWUKD	KF-958	
	MSWUKE	KF-959	
	MSWUKF	KF-960	
	UK03	KF-1043 A	
	UK06,90	KF-1045	
	UK07	KF-1037	
	UK10	KF-1044	

Museum number	Salvage number	Field/oceanaria number	Name
CAS 23277	M-293		
	UK16or91	KF-1032	
	UK33	KF-1041	
	UK42	KF-1042	
	UKKF08*	KF-1040	

APPENDIX C

PROCEDURES FOR PROCESSING MANATEE BONE SAMPLES

Details are provided below on methods developed and employed for preparation of bone samples. Background information and cautions are provided as necessary to allow future users of this guide to avoid procedural errors that could otherwise result in loss of material or time.

Collecting

The earbone is collected by removing the head and dislodging the lower jaw. The auditory apparatus in manatees consists essentially of three parts (Kaiser, 1974; Petit, 1955; Robineau, 1969) (see Fig. 2.3): a) the group of ossicles (incus, malleus, and stapes); b) the tympanic bone; and c) the periotic bone, with its pars petrosa, pars mastoidea, and the dome (tholus or tegmen tympani). The dome is the portion by which the complex abuts the squamosal bone in the skull. The tympano-periotic complex is extracted by removing the flesh around it and applying some leverage with a necropsy tool. Caution should be taken to remove the whole complex, given that the tympanic bone tends

to break loose. Special attention should also be given to recover the ossicles.

Fixation and Storage

Samples should be placed in a fluid medium at the time of necropsy or as soon afterward as possible. This preserves the integrity of the bone, prevents brittleness and allows the production of microscopic slides that are superior to those from samples that have been stored dry. Neutral-buffered formalin (10%) constitutes an appropriate medium, but 1:1 mixtures of glycerin:70% alcohol or glycerin:distilled water may also be used (D. Odell, pers. comm.). Conventional fixatives partially decalcify bones, and Frost (1983) prefers absolute acetone or 70% alcohol for human bone. Long-term storage in formalin should particularly be avoided in tetracycline-labeled specimens because it destroys the label by causing it to leach, although phosphate buffered formalin may preserve it (Frost, 1983).

Rough Sectioning

Attempts to section bone were made using a band saw, a hack saw and a rock saw. The band saw did not use water as a lubricating material and tended to burn the tissue. The hack saw produced good cuts but required a large amount of time. Best results (both in terms of surface smoothness and

time consumed) were obtained with the rock saw working under a mixture of water and oil. A diamond-tipped steel blade produces adequate cuts for this type of study. A finer silicon carbide cutting wheel requires more time and skill, but produces an even surface and less loss of bone tissue.

Figs. 2.2 and 2.3 illustrate the various bones and planes of section used in this study. Recommended location of cut for the preferred bone (dome portion of the periotic bone) is shown in Fig. 2.3A2. The central part of the periotic dome constitutes the choice area for processing. Although the layers are present throughout the dome, they become more compressed towards the extremities, impairing the reading of microscopic slides. The tympanic bone should be sectioned in the central portion (Fig. 2.3A1), and layers counted on the outer, more pointed area. Layers tend to run closer to the edge and eventually disappear as they move opposite to the outer point.

Isomet Sectioning

The ISOMET² low-speed saw manufactured by Buehler Ltd. (proven very satisfactory for sectioning dolphin teeth) was employed to obtain thick (140 μ m) sections of manatee bone from the rough-cut sections. Thin, rigid circular, high-concentration diamond wafering blades (4 and 5 inch sizes)

²The use of any trade names in this document does not constitute an endorsement of any product, product line, or company.

were used in conjunction with two sizes of flanges (1 3/4" and 1 3/8" diameter) to allow maximum depth of cut. Rotation of the specimen without altering the original direction of the cut increased the cutting depth. Small pieces of bone were clamped directly to one of 3 accessory chucks provided by the manufacturer. Larger bones were attached to either a wafer-type or a wooden chuck by means of thermoplastic cement melted over a Bunsen burner. Weights up to 75 g were used on the shaft, depending on the size of the bone being cut. The saw was never operated at a speed higher than 5 (speed control range 1-10). The blade was kept from overheating by immersion in a lubricating pan. Tap water with a drop of household detergent was used to break the surface tension in the pan.

The 4-mm rough section may also be obtained by sawing the bone directly on the rock saw, representing a considerable savings in time. A thin rock-saw blade should be used to prevent extreme pressure on the bone. Using the ISOMET instead of the rock saw is not recommended when dealing with brittle specimens. Additionally, the rock saw cannot substitute for the ISOMET in obtaining a "thick section" for ultraviolet-light examination.

Formalin Fixation

For formalin-fixation, 4-mm sections were placed in clear plastic boxes (2.8 x 2.8 x 2.8 cm) with hinged lids.

All sides were perforated with a hot probe to allow the free flow of fluids. Larger specimens were placed in larger plastic boxes (5.1 x 5.1 x 1.9 cm), plastic bags with holes, or in cotton or panty-hose bags. Bond paper (100%) was used to label the sections. Fixatives usually penetrate less than 0.5 cm in 24 hours, and therefore the sections did not exceed these dimensions. Long periods of formalin fixation are not recommended for specimens that have been labeled with tetracycline. Formalin eventually decalcifies bone, and because tetracycline is bound to the calcium, it will be leached. Rat bone loses the tetracycline label within 48 hours of fixation in formalin (T. Wronski, pers. comm.).

Boxes were placed overnight in a large jar containing 10% neutral-buffered formalin. The jar was shaken periodically to allow all surfaces to be in contact with the fixative. Sections were rinsed under tap water for approximately 6 hours before decalcification.

Decalcification

Bone is a difficult tissue for histological work. It is therefore common practice to soften bone by dissolving its mineral constituents, rendering it suitable for cutting in the microtome. The minerals are removed by immersion in RDO¹, a rapid decalcifying agent containing hydrochloric acid as the principal active ingredient. The acid reacts with calcium in mineralized tissues to form soluble calcium

salts. A more appropriate term for this process, however, would be demineralization, because the acid removes not only calcium but any other mineral present in bone in smaller concentrations (Anderson, 1982). The process does not alter the organic framework. However, decalcifying agents remove tetracycline labels (Frost et al., 1961; Nielsen, 1972). It is advisable to use bone sections no thicker than 4 mm in the decalcification process. The larger the specimen, the longer the decalcification time. Thicker samples may also produce unequal decalcification and favor tissue damage on the surfaces due to continued exposure to the decalcifying agent.

Boxes or bags containing bone samples were placed in a jar containing RDO. A 20:1 volume ratio of RDO to tissue was employed. The jar was agitated by hand every 1-3 hours to increase circulation. Decalcification time varies with the size and density of the specimen. On average it takes 10-12 hours for a 4 mm section to become flexible and slightly translucent. Small cancellous bones may be decalcified in 4 hours. Completion of the process may be determined by: 1) assessing the flexibility of the tissue from time to time; 2) poking the inner surface gently with a probe; or 3) proceeding with a chemical or x-ray analysis. Method 1 was employed with care due to the risk of breaking the piece; method 2 may damage the specimen by producing holes in the tissue; chemical analyses were never tried; x-

rays provide a quick and definitive answer, but incur additional expenses. Over-decalcification in RDO causes damage and renders the bone useless for age determination. The RDO may be reused a few times, but renewal of the fluid is necessary when it loses its strength. When decalcification was judged complete, the boxes were removed from RDO and rinsed in running tap water for at least 3 hours to neutralize the action of the acid. Complete removal of acid from the bone is necessary to prevent RDO from interfering with the stain.

Storage subsequent to decalcification is not recommended. If unavoidable, sections may be placed in glycerin or in distilled water under refrigeration, but storage longer than a few days should be avoided. Formalin must not be used for storage. Embedding techniques do not seem feasible, given the very compact structure of the bone. Celloidin embedding is performed in human earbones, after they have been decalcified, but this is an extremely time-consuming process.

Microtome Sectioning

Thin sections (40 μm) were cut in a sledge-type freezing microtome. Some sections were obtained from a Lipshaw² 80A microtome with movable stage and stationary AO-950, 250-mm blade. However, better sections were obtained with an AO Reichert² Sliding Microtome Model 860 equipped

with a wedge-shaped Reichert-Jung² 945 Microtome Knife (185 mm, C Profile). A knife-blade set at about a 65-degree angle to the long axis of the bone was employed following Perrin and Myrick (1980).

Freezing was obtained either through CO₂-control or by placing alcohol and dry ice on troughs with a larger stage. The specimen was fastened to the stage by means of a small platform of frozen-tissue embedding medium (Histo Prep³).

The tissue was sectioned at 40 μ m. This thickness allowed obtaining of complete sections and still obtain satisfactory resolution of layers under the microscope. Sections were returned to the plastic boxes and rinsed in tap water for approximately 6 hours to remove any residual RDO.

Staining

Mayer's hematoxylin (modified by Helen Grue) was used to stain bone sections (Myrick et al., 1983). The stain was prepared by dissolving 1.0 g of hematoxylin in 1.0 l of distilled water, using low heat as necessary; 0.1 g of sodium iodate was added to the mixture, followed by 50.0 g of aluminum potassium sulfate (alum), stirring until the alum was dissolved. Stain was stored under refrigeration but may be used immediately after preparation. Specimens were stained in the same boxes used for decalcification.

Staining worked best at room temperature. Staining time varies with age of the stain. It is advisable to check periodically under the microscope for contrast. Experience showed that a range from 30 to 60 minutes provided good contrast. Excess stain was removed by a tap-water rinse, until the water ran clear. To enhance the contrast of stained layers, the thin sections were "blued" in a 0.5% solution of ammonia water for up to 1 minute. However, prolonged exposure to the ammonia will reverse the stain. After blueing, the sections were rinsed in tap water for 3-5 minutes.

Glycerin Exchange and Mounting

Stained thin sections retain their color best when mounted in pure glycerin. Thin sections and labels were transferred from the container to a jar containing a 1:1 solution of glycerin and distilled water and soaked for 5-10 minutes. They were then placed in a jar of 100% glycerin overnight to complete the exchange of glycerin for water in the thin sections, as recommended by Myrick et al. (1983).

One to three of the best sections were arranged on a slide with coverslips. Edges of coverslips were sealed with histological mounting media (Permout, Eukitt or Tissue-Tek)². Sections destined for viewing under UV light were mounted directly on the slide and sealed with Fluormount².

General Comments

The technique is both labor- and time-consuming. The entire process takes over 24 hours to complete, from fixation of the sample to examination of the slide. The procedure can be accelerated by processing several pieces at the same time, or carrying some of the stages overnight (such as formalin and glycerin fixation). The ISOMET cutting step may be bypassed by using a thin rock-saw blade. With care, the 4-mm slab may be obtained directly from the rock saw, but special attention must be taken when processing brittle museum specimens. Stained sections need not be differentiated and the glycerin method saves the steps of running the bone through the alcohols.

APPENDIX D

TERMINOLOGY RELATED TO BONE STRUCTURE AND DESCRIPTIONS OF BONES EXAMINED FOR PRESENCE OF GROWTH-LAYER GROUPS

Terminology

Bone is a highly specialized type of connective tissue that forms the skeletal framework of most vertebrates and constantly renews itself to meet both mechanical and metabolic demands. Mechanical properties are most widely recognized, but bone also has protective, hematopoietic and metabolic functions. For further discussion on the biology of bone and for derivation of the terminology given below consult references by Albright and Brand (1987), Hancox (1972), Jee (1983), Martin and Burr (1989), McLean and Urist (1968), and Vaughan (1970).

Mineralized bone consists of 76% inorganic material and 24% organic matrix. The organic matrix is the extracellular organic phase, composed of protein, glycoprotein, and polysaccharide. It consists primarily of collagenous fibers embedded in an amorphous ground substance. The mineral content (inorganic matrix) is mainly represented by

insoluble calcium phosphate deposited in and around the collagen fibers.

Osteoblasts are bone-forming cells that synthesize and secrete unmineralized bone matrix, the osteoid. Osteocytes are the principal cells of fully formed bones. They are entrapped in lacunae and derive from osteoblasts that have secreted bone around themselves. Osteoclasts are multinucleated giant cells responsible for the resorption of bone.

Reticular vascular bone is nonlamellar and characterized by the random orientation of its loosely packed collagen fibers and lacunae. It is permeated by primary osteons that provide for the vascular appearance. Primary osteons have 1 to 3 lamellae and rounded osteocytes.

Bones of adult mammals consist of two basic structures: compact (also known as hard, or cortical), and cancellous (also known as spongy, or trabecular). Whether compact or cancellous, adult mammalian bone is lamellated.

Lamellations are stacks of parallel or concentrically curved sheets or lamellae, where collagen fibers are oriented parallel to each other. Small cavities (lacunae) are regularly spaced throughout lamellar compact and cancellous bone. Lacunae are connected by thin tubular channels (canaliculi). Bone cells (osteocytes) and their long cytoplasmic processes occupy the lacunae and canaliculi, respectively. Cortical bone is primarily a dense solid

mass, and forms the outer wall or "shell" of all bones.

Trabecular bone consists of a network of fine interlacing partitions ("trabeculae") enclosing cavities that contain either red or fatty marrow (75% of its volume).

Lamellae arranged concentrically around a longitudinal vascular channel form an osteon, or Haversian system. Larger canals have an irregular edge (resorption cavities), where active removal of both mineral and matrix may be occurring as osteoid is being laid. Osteons are the main structural unit of cortical bone. Haversian canals communicate with the periosteum, bone marrow, and each other through transverse or oblique channels (Volkman's canals), which can be differentiated from Haversian canals by the lack of concentric lamellae. A layer of mineralized matrix deficient in collagen fibers (cement line) surrounds the border of each osteon. Reversal lines are irregular and scalloped, occurring after bone removal, or resorption. Primary osteons do not have cement lines (are not produced by remodeling), have smaller vascular channels and fewer lamellae than secondary osteons.

Adult cancellous bone is a network of anastomosing trabeculae with intertrabecular spaces containing bone marrow. Trabeculae do not contain blood vessels. Trabeculae packets are the bone structural units of cancellous bone.

The periosteal surface is the outer boundary of adult bone, between the outer circumferential lamellae and the periosteum.

The periosteum is the connective tissue surrounding bone. The endosteum lines the walls of bone marrow cavities and the Haversian canals of compact bone, and covers trabeculae of cancellous bone.

Bone grows by appositional growth due to the nonexpandable nature of mineralized bone tissue. Bone resorption involves the initial release of calcium and phosphate ion into the extracellular fluid and the removal of the organic matrix. Osteoclastic resorption predominates, although some resorption is also performed by osteocytes.

Plexiform bone is named for the appearance of intertwining vascular plexuses throughout the bone. Structurally it is like highly oriented cancellous bone, the plates of which thicken by surface bone apposition to become dense cortical bone. Rapidly deposited, plexiform bone is commonly seen in larger, rapidly growing animals (e.g., artiodactyls, elephants, some dinosaurs), but also in larger breeds of dogs as well as human jaws.

Descriptions of Manatee Bones

Stapes

The central part of the bone consists of a large core of primary reticular vascular bone. The band immediately following the core material is wide relative to the subsequent bands and contains secondary reticular bone. In histological preparations, the primary and secondary vascular bone usually break apart from the compact bone. Volkman's canals are more abundant and primary canals less abundant in this area. The transition into compact bone occurs without a sharp demarcation, with a reduced number of canals. There is no white rim or conspicuous first-year zone, and the next change is limited by a well-defined adhesion line (Fig. D.1).

Ribs

Manatee ribs are fusiform. The middle of the shaft is the thickest part (Fig. 2.2B). The ribs of adult Florida manatees continue to grow in thickness by apposition of new bone (Fawcett, 1942a, 1942b). Therefore, their outer border has a curved profile, whereas the pleural side is flattened in cross-section.

Enlow and Brown (1958) and Fawcett (1942a, 1942b) have called attention to the massiveness of manatee ribs, absence of a medullary cavity, and lack of cancellous or spongy

bone, at least in the shaft of the ribs. Spongiosa is present in rib sections proximal to the costal cartilage; this trabecular material disappears distally. According to Fawcett (1942a, 1942b) successive layers of compact bone interpose between the spongiosa and the periosteum of the outer curvature. Microscopically the rib has a characteristic appearance. Small (2-3 rings per osteon) primary osteons are arranged in distinctive rows and surrounded by interstitial non-lamellar bone. Secondary lamellar bone is also present, irregularly distributed, and containing flattened osteocytes. The pleural surface is commonly characterized by erosion of the compact layers spreading outward as a diffuse, centrifugal, resorptive front (Fig. D.2). Layers at this surface are scalloped in appearance, suggesting modeling resorption. Some sections contain many large erosion bays, giving the bone a porous appearance. Ribs present high remodeling rates because they are cyclically loaded by respiratory muscles (Martin and Burr, 1989). The depositing outer surface is smooth, and layers may be observed on the periosteal portion, especially at the corners of the oval cross-sections, where the crescentic layers are narrowest (Fig. D.3A). The outer surface is composed of plexiform bone, composed of a symmetrical, three-dimensional plexus of primary canals (Enlow, 1963; Enlow and Brown, 1958) (Fig. D.3B). Plexiform bone can be more easily observed in sections of young

animals, where resorption has not yet interfered with the tissue.

Long bones

Long bones have been useful in some age determination studies, especially with small species (Lemnell, 1973). Manatee humeri, radii, and ulnae were sectioned at both extremities and mid-shaft (Figs. 2.2D, 2.2E, 2.2F). Coarse-cancellous bone was generally more extensively distributed in the proximal and distal thirds of long bones. The periosteal surface contains numerous Haversian canals remodeling the layers, most of them with a scalloped appearance (Fig. D.4).

Phalanges, Carpal, Hyoid and Pelvic Bones

These bones have the general structure of Haversian tissue (Fig. D.5). The deeper core is composed of cancellous bone or primitive trabecular bone (Fig. D.6). Periosteal bone is restricted to the edge of the bone (Fig. D.7), where scalloped layers are difficult to distinguish due to a high rate of resorption.

Mandible

Mandibles have been successfully used for age determination in insectivores, lagomorphs, rodents, fissipeds, and primates (Castagnet, 1980; Klevezal' and

Kleinenberg, 1969). Layers have been demonstrated in mandibles of odontocetes (Laws, 1960; Nishiwaki et al., 1961), but not in mysticetes (Klevezal' and Kleinenberg, 1969). Layers in mandibles of cetaceans may not correspond to annuli in all cases (Brodie, 1969; Hay, 1980; Watts and Gaskin, 1989). Layers are present in mandibles of dugongs but may not correspond to annuli (Marsh, 1980; Mitchell, 1978). Layers of unknown temporal significance have been reported for mandibles of two Florida manatees (Domning and Myrick, 1980).

Manatee mandibular tissue is composed of plexiform tissue (Fig. D.8). The mandibular condyle is an important center of growth with movement in general in a posterior direction. The lingual surfaces of the sections examined exhibited an area of active resorption and the postero-central area consisted of spongy tissue. One or two scalloped lines representing resorption (Fig. D.9) were seen at the angle of the jaw of 5 specimens examined. These lines were not considered to be growth-layer groups.

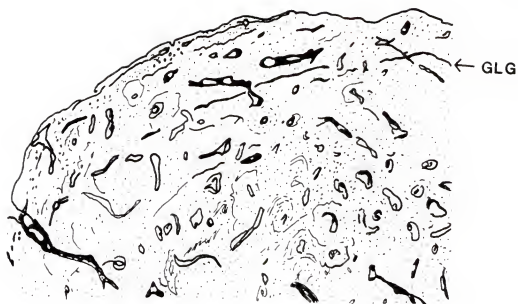


Figure D.1. Thin section of the stapes of UF 23993, showing the vascular bone (VB) and growth-layer groups (1-4). Original magnification 20x.

Figure D.2. General view (original magnification 10x) and detailed thin section (original magnification 20x) of a rib of UF 20758. Note the Haversian systems (HS) and the scalloped layers on the pleural surface (PS). The outer surface (OS) is composed of plexiform bone).



Figure D.3. Details of a thin section of rib of UF 20758 depicted on Fig. D.2. A, growth-layer group (GLG) on the pleural surface; B, plexiform bone (PB) and Haversian systems (HS) in the core. Original magnification 40x.



A



B



Figure D.4. Thin section of the radius of SWFTm-8646B. Note the extensive effect of Haversian systems (HS) obliterating the periosteal growth-layer groups (GLG). Original magnification 20x.

Figure D.5. General view (original magnification 10x) and detail of thin section (original magnification 40x) of the hyoid bone of KDL-8827. Note the large amount of Haversian systems (HS) obliterating older growth-layer groups (GLG).

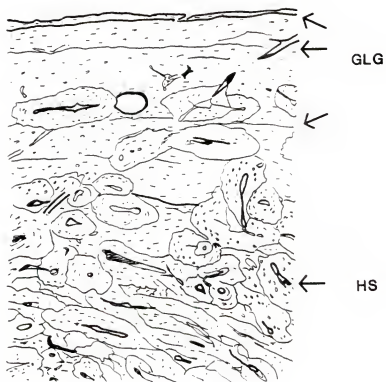


Figure D.6. Thin section of the pelvic bone of UF 20773.
A, general view (original magnification 10x); B,
details of the cancellous bone core (original
magnification 100x); C, details of the periosteal
portion, showing extensive resorption and scalloped
lines (original magnification 100x).

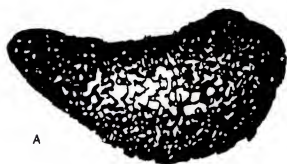


Figure D.7. General view (original magnification 10x) and detail of thin section (original magnification 40x) of the carpal bone of KDL-8827. Note the periosteal growth-layer groups (GLG) undergoing resorption by Haversian systems (HS), and a large erosion bay (EB).

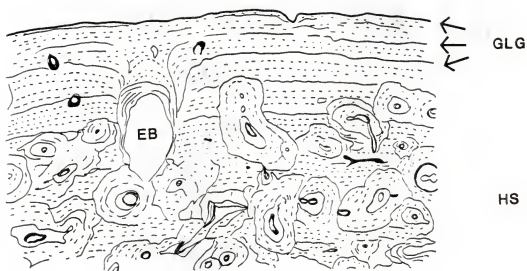
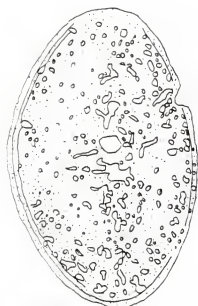


Figure D.8. Thin section of the mandible of UF 20757. Note the plexiform bone (PB) and Haversian systems (HS) of the core. No growth-layer groups are present. Original magnification 20x.



PB

HS

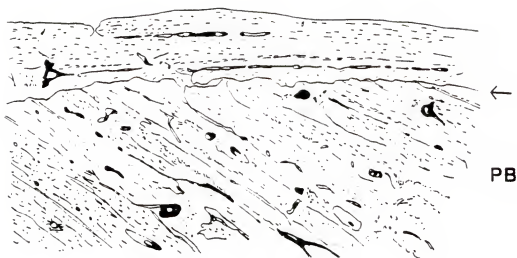


Figure D.9. Detail of thin section of the mandible of UF 20757. Note the presence of plexiform bone (PB) and scalloped line (arrow), not considered to be a growth-layer group. Original magnification 40x.

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BIOGRAPHICAL SKETCH

Miriam Marmontel was born on July 9, 1958, in Porto Alegre, in the Brazilian gaucho lands. Between summers at the beach and weekends in the hills around the house, she learned to appreciate nature and wildlife. Miriam graduated from Universidade do Rio Grande in 1981, with a double major in biological and geological Oceanography. After working a few years from the southern coastal plain to eastern Amazonia, she entered the University of Miami's Rosenstiel School of Marine and Atmospheric Science and obtained her M.Sc. in Marine biology with a thesis on manatee reproduction in 1988. Even before defending her thesis, she was taking classes at the University of Florida. Manatees continued in her mind and heart, and she graduated in the spring of '93 after 4.5 years in Gainesville. She is looking forward to returning to the jungle and continuing work with mamirauás.


I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


Stephen R. Humphrey, Chair
Professor of Forest Resources
and Conservation

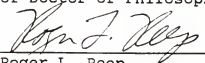
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Thomas J. O'Shea, Cochair
Assistant Professor of Forest
Resources and Conservation

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John F. Eisenberg
Katharine Ordway Professor of
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.


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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Melvin E. Sunquist
Associate Scientist of
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Conservation

This dissertation was submitted to the Graduate Faculty of the School of Forest Resources and Conservation in the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

May, 1993

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